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(21) International Application Number: PCT/US97/06293 (22) International Filing Date: 15 April 1997 (15.04.97) (30) Priority Data: 08/632,575 15 April 1996 (15.04.96) US (71) Applicant: PROMEGA CORPORATION [US/US]; 2800 Woods Hollow Road, Madison, WI 53711-5399 (US). (72) Inventors: SCHUMM, James, W.; 5843 Timber Ridge Trail, Madison, WI 53711 (US). MICKA, Katherine, A.; 1842 Paddock Place, Oregon, WI 53575 (US). RABBACH, Dawn, R.; 6649 Wendell Way, DeForest, WI 53532 (US). (74) Agents: FRENCHICK, Grady, J. et al.; Stroud, Stroud, Willink, Thompson & Howard, Suite 300, 25 West Main Street, P.O. Box 2236, Madison, WI 53701-2236 (US).		(81) Designated States: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TT, UA, UG, UZ, VN, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(54) Title: MULTIPLEX AMPLIFICATION OF SHORT TANDEM REPEAT LOCI (57) Abstract The invention is directed to the simultaneous amplification of multiple distinct genetic loci using PCR or other amplification systems to determine in one multiplex reaction the alleles of each of the loci contained in the multiplex reaction. The genetic loci analyzed comprise the following: HUMvWFA31, HUMLIPOL, HUMBFXIII, HUMF13A01, HUMFESFPS, HUMTH01, HUMTPOX, HUMCSF1PO, D22S683, D20S481, D19S253, D17S1299, D17S1298, D16S753, D16S539, D16S490, D14S562, D14S548, D14S118, D13S317, D10S1239, D9S930, D7S820, D5S818, D4S2368, D3S1539.		

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MULTIPLEX AMPLIFICATION OF SHORT TANDEM REPEAT LOCI

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part of United States patent application Serial Number 08/316,544, filed September 30, 1994. The
5 entire disclosure of that parent application is incorporated by reference herein.

STATEMENT REGARDING FEDERALLY SPONSORED
RESEARCH OR DEVELOPMENT

Not Applicable

10 BACKGROUND OF THE INVENTION

The present invention is generally directed to the detection of genetic markers in a genomic system. The present invention is more specifically directed to the simultaneous amplification of multiple distinct polymorphic genetic loci using the polymerase chain reaction or other amplification
15 systems to determine in one reaction the alleles of each locus contained within the multiplex system.

In recent years, the discovery and development of polymorphic short tandem repeats (STRs) as genetic markers has stimulated progress in the development of linkage maps, the identification and characterization of
20 diseased genes, and the simplification and precision of DNA typing.

Many loci, at least in the human genome, contain polymorphic STR regions (Adamson, D., et al. (1995) "A collection of ordered tetranucleotide-repeat markers from the human genome," *Am. J. Hum. Genet.* 57: 619-628; Murray, J. C., et al. (1994) "A comprehensive human linkage map
25 with centimorgan density," *Science* 265: 2049-2054; Hudson, T. J., Engelstein, M., Lee, M. K., Ho, E. C., Rubenfield, M. J., Adams, C. P.,

Housman, D. E., and Dracopoli, N. C. (1992) "Isolating and chromosomal assignment of 100 highly informative human simple sequence repeat polymorphisms," *Genomics* 13: 622-629). STR loci consist of short, repetitive sequence elements of 3 to 7 base pairs in length. It is estimated
5 that there are 2,000,000 expected trimeric and tetrameric STRs present as frequently as once every 15 kilobases (kb) in the human genome (Edwards et al. (1991) "DNA typing and genetic mapping with trimeric and tetrameric tandem repeats." *Am. J. Hum. Genet.* 49: 746-756; Beckman, J. S., and Weber, J. L. (1992) "Survey of human and rat microsatellites," *Genomics*
10 12: 627-631). Nearly half of the STR loci studied by Edwards et al. (1991) are polymorphic, which provides a rich source of genetic markers.

Variation in the number of short tandem repeat units at a particular locus causes the length of the DNA at that locus to vary from allele to allele and from individual to individual. Such length polymorphism is reminiscent
15 of variable number of tandem repeats (VNTR) loci (Nakamura, Y., et al. (1987) "Variable number of tandem repeat (VNTR) markers for human gene mapping," *Science* 235: 1616-1622) and minisatellite loci (Jeffreys, A. J., et al. (1985) "Hypervariable 'minisatellite' regions in human DNA," *Nature* 314: 67-73), both of which contain considerably longer repeat units than
20 STR loci. Such length polymorphism is also reminiscent of the dinucleotide repeat form of microsatellite loci (Litt, M. and Luty, J. A. (1989) "A hypervariable microsatellite revealed by in-vitro amplification of a dinucleotide repeat within the cardiac muscle actin gene," *Am. J. Hum. Genet.* 44: 397-401, Tautz, D., et al. (1986) "Cryptic simplicity in DNA is
25 a major source of genetic variation," *Nature* 322: 652-656, Weber, J. L. and May, P. E. (1989) "Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction," *Am. J. Hum. Genet.* 44: 388-396; Beckmann and Weber, (1992)), a form of microsatellite loci with shorter repeat units than STR loci.

30 Polymorphic STR loci are extremely useful markers for human identification, paternity testing and genetic mapping. STR loci may be

amplified via the polymerase chain reaction (PCR) by employing specific primer sequences identified in the regions flanking the tandem repeat.

Alleles of these loci are differentiated by the number of copies of the repeat sequence contained within the amplified region and are distinguished from one another following electrophoretic separation by any suitable detection method including radioactivity, fluorescence, silver stain, and color.

To minimize labor, materials and analysis time, it is desirable to analyze multiple loci and/or more samples simultaneously. One approach for reaching this goal involves amplification of multiple loci simultaneously in a single reaction. Such "multiplex" amplifications, as they are called, have been described extensively in the literature. Multiplex amplification sets have been extensively developed for analysis of genes related to human genetic diseases such as Duchenne Muscular Dystrophy (Chamberlain, J. S., et al. (1988) "Deletion screening of the Duchenne muscular dystrophy locus via multiplex DNA amplification," *Nucleic Acid Res.* 16: 11141-11156; Chamberlain, J.S., et al. (1989), "Multiple PCR for the diagnosis of Duchenne muscular dystrophy," In *PCR Protocols, A Guide to Methods and Application* (ed. Gelfand, D. H., et al.) pp. 272-281. Academic Press, San Diego, CA; Beggs, A. H., et al. (1990) "Detection of 98% DMD/BMD gene deletions by PCR," *Hum. Genet.* 86: 45-48; Clemens, P. R., et al. (1991). "Carrier detection and prenatal diagnosis in Duchenne and Becker muscular dystrophy families, using dinucleotide repeat polymorphisms," *Am J. Hum. Genet.* 49: 951-960; Schwartz, J. S., et al. (1992) "Fluorescent multiple linkage analysis and carrier detection for Duchenne/Becker's muscular dystrophy," *Am J. Hum. Genet.* 51: 721-729; Covone, A. E., et al. (1992) "Screening Duchenne and Becker muscular dystrophy patients for deletions in 30 exons of the dystrophin gene by three-multiplex PCR," *Am. J. Hum. Genet.* 51: 675-677), Lesch-Nyhan Syndrome (Gibbs, R. A., et al. (1990) "Multiple DNA deletion detection and exon sequencing of the hypoxanthine phosphoribosyltransferase gene in Lesch-Nyhan families,"

Genomics 7: 235-244), Cystic Fibrosis (Estivill, X., et al. (1991) "Prenatal diagnosis of cystic fibrosis by multiplex PCR of mutation and microsatellite alleles," *Lancet* 338: 458; Fortina, P., et al. (1992) "Non-radioactive detection of the most common mutations in the cystic fibrosis transmembrane conductance regulator gene by multiplex polymerase chain reaction," *Hum. Genet.* 90: 375-378; Ferrie, R.M., et al. (1992) "Development, multiplexing, and application of ARMS tests for common mutations in the CFTR gene," *Am. J. Hum. Genet.* 51: 251-262; Morral, N. and Estivill, X. (1992) "Multiplex PCR amplification of three microsatellites within the CFTR gene," *Genomics* 51: 1362-1364), and Retinoblastoma (Lohmann, D., et al. (1992) "Detection of small RB1 gene deletions in retinoblastoma by multiplex PCR and high-resolution gel electrophoresis," *Hum. Genet.* 89: 49-53). Multiplex amplification of polymorphic microsatellite markers (Clemens et al. (1991); Schwartz et al. (1992); Huang, T. H.-M., et al. (1992) "Genetic mapping of four dinucleotide repeat loci DXS435, DXS45, DXS454, DXS424, on the X chromosome using the multiplex polymerase chain reaction," *Genomics* 13: 375-380) and even STR markers (Edwards, A., et al. (1992) "Genetic variation at five trimeric and tetrameric tandem repeat loci in four human population groups," *Genomics* 12: 241-253; Kimpton, C. P., et al. (1993) "Automated DNA profiling employing multiplex amplification of short tandem repeat loci," *PCR Methods and Applications* 3: 13-22; Hammond, H. A., et al. (1994) "Evaluation of 13 STR loci for use in personal identification applications," *Am. J. Hum. Genet.* 55: 175-189; Schumm, J. W. et al. (1994) "Development of nonisotopic multiplex amplification sets for analysis of polymorphic STR loci," in "The Fourth International Symposium on Human Identification 1993," pp. 177-187; Oldroyd, N. J., et al. (1995) "A highly discriminating octoplex short tandem repeat polymerase chain reaction system suitable for human individual identification," *Electrophoresis* 16: 334-337) have been described.

These amplified products are generally separated by one of several methods of electrophoresis known to those skilled in the art. Several well-known methods of detection of the amplified products have also been described. While ethidium bromide staining or silver staining of amplified fragments is employed in some cases, in others it is preferred to use methods which label only one of the two strands of the amplified material. Examples of this include radioactive or fluorescent labeling of one of the two primers prior to the amplification of a locus. One of the more sophisticated approaches to detection is the use of different fluorescent labels to allow detection of amplified materials representing different loci, but existing in the same space following electrophoresis. The products of the different loci are differentiated with the use of filters or other discriminating detectors, which allow visualization of one fluorescent label at a time.

Reference is made to International Publications WO 93/18177 and WO 93/18178 to Fortina et al., which are directed to methods and kits for diagnosing diseases such as Cystic Fibrosis and β -thalassemia, respectively, using an allele-specific multiplex polymerase chain reaction system. According to Fortina et al., multiplex PCR has also been used for simultaneous amplification of multiple target sequences, permitting mutant allele scanning using two lanes of an agarose gel.

Ballabio, A. et al. (1991) "PCR Tests for Cystic Fibrosis Deletion," *Nature*, 343: 220, disclose a single-tube, multiplex allele-specific PCR test using two different dye-tagged fluorescent primers for detection of the F508 cystic fibrosis mutation.

While there are multiplex amplification procedures for specific loci, the use of multiplex amplification procedures is greatly desired for the detection of alleles in other types of loci such as specific STR loci. It is also desirable to identify primers which make multiplex amplification of such loci possible.

BRIEF SUMMARY OF THE INVENTION

It is, therefore, an object of the present invention to provide a method and materials for the simultaneous amplification of multiple distinct polymorphic short tandem repeat (STR) loci using PCR or other amplification systems to determine, in one reaction, the alleles of each locus contained within the multiplex. Multiplex analysis of the sets of specific STR loci disclosed herein have not been previously described in the prior art. There has also not been any previous description of the sequences for many of the primers disclosed herein below, all of which are shown to be useful for multiplex amplification of such STR loci.

It is also an object of the present invention to provide a method, a kit, and primers specific for multiplex amplifications comprising specified loci.

These and other objects are addressed by the present invention which is directed to a method and materials for simultaneously analyzing or determining the alleles present at each individual locus of each multiplex. In general, the method of this invention comprises the steps of (a) obtaining at least one DNA sample to be analyzed, wherein the DNA sample has at least two loci which can be amplified together; (b) amplifying the STR sequences in the DNA sample; and (c) detecting the amplified materials in a fashion which reveals the polymorphic nature of the systems employed.

More specifically, the method of this invention is a method of simultaneously determining the alleles present in at least three tandem repeat loci from one or more DNA samples, such method comprising the steps of:

- (a) obtaining at least one DNA sample to be analyzed, wherein the DNA sample has a set of at least three loci which can be amplified together, wherein the set of loci is selected from a specific group or sets of loci disclosed herein;

(b) co-amplifying the set of loci in a multiplex amplification reaction, wherein the product of the reaction is a mixture of amplified alleles from each of the co-amplified loci in the set; and

5 (c) evaluating the amplified alleles in the mixture to determine the alleles present at each of the loci analyzed in the set within the DNA sample.

In one embodiment of this invention, three STR loci are amplified together, and the set of three co-amplified loci is selected from the group of sets consisting of:

10 D3S1539, D19S253, D13S317;
D10S1239, D9S930, D20S481;
D10S1239, D4S2368, D20S481;
D10S1239, D9S930, D4S2368;
D16S539, D7S820, D13S317; and
15 D10S1239, D9S930, D13S317.

In a more preferred embodiment of the method of this invention, the DNA sample has at least four STR loci which can be amplified together, and the set of co-amplified loci is selected from the group of loci consisting of:

20 D3S1539, D4S2368, D5S818, D7S820, D9S930, D10S1239,
D13S317, D14S118, D14S548, D14S562, D16S490, D16S539,
D16S753, D17S1298, D17S1299, D19S253, D20S481, D22S683,
HUMCSF1PO, HUMTPOX, HUMTH01, HUMFESFPS, HUMF13A01,
HUMBFXIII, HUMLIPOL, HUMvWFA31.

The set of at least four STR loci amplified together is more preferably a set
25 selected from the sets of four loci comprising:

D3S1539, D7S820, D13S317, D5S818;
D17S1298, D7S820, D13S317, D5S818;
D20S481, D7S820, D13S317, D5S818;
D9S930, D7S820, D13S317, D5S818;
30 D10S1239, D7S820, D13S317, D5S818;
D14S118, D7S820, D13S317, D5S818;

D14S562, D7S820, D13S317, D5S818;
D14S548, D7S820, D13S317, D5S818;
D16S490, D7S820, D13S317, D5S818;
D17S1299, D7S820, D13S317, D5S818;
5 D16S539, D7S820, D13S317, D5S818;
D22S683, D7S820, D13S317, D5S818;
D16S753, D7S820, D13S317, D5S818;
D3S1539, D19S253, D13S317, D20S481;
D3S1539, D19S253, D4S2368, D20S481;
10 D10S1239, D9S930, D4S2368, D20S481; and
D16S539, D7S820, D13S317, HUMvWFA31.

More preferably, the set of STR loci amplified together is a set of six loci, selected from the sets of loci comprising:

D16S539, D7S820, D13S317, D5S818, HUMCSF1PO, HUMTPOX;
15 and

D16S539, D7S820, D13S317, D5S818, HUMF13A01, HUMFESFPS.
Yet more preferably, the set of STR loci amplified together is a set of seven loci, selected from the sets of loci comprising:

D16S539, D7S820, D13S317, D5S818, HUMCSF1PO, HUMTPOX,
20 HUMTH01; and

D16S539, D7S820, D13S317, D5S818, HUMF13A01, HUMFESFPS,
HUMBFXIII.

Even more preferably, the set of STR loci amplified together is a set of eight loci, selected from the sets of loci comprising:

D16S539, D7S820, D13S317, D5S818, HUMCSF1PO, HUMTPOX,
25 HUMTH01, HUMvWFA31; and

D16S539, D7S820, D13S317, D5S818, HUMF13A01, HUMFESFPS,
HUMBFXIII, HUMLIPOI.

The multiplex amplification reaction step of the method is preferably
30 done using a pair of primers flanking each locus in the set of loci co-amplified in the reaction. More preferably, pairs of primers are selected for

the multiplex amplification reaction which produce alleles from each locus that do not overlap the alleles of the other loci in the set co-amplified therein, when the alleles are separated by gel electrophoresis. Even more preferably, the sequence of one of each pair of primers used in the multiplex amplification reaction is selected from a group of primer sequences consisting of:

SEQ ID NO:1 and SEQ ID NO:2, when one of the loci in the set is D7S820;

SEQ ID NO:3 and SEQ ID NO:4, when one of the loci in the set is D13S317;

SEQ ID NO:5 and SEQ ID NO:6, when one of the loci in the set is D5S818;

SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:49, when one of the loci in the set is D3S1539;

SEQ ID NO:9, SEQ ID NO:10, when one of the loci in the set is D17S1298;

SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:52, SEQ ID NO: 53, when one of the loci in the set is D20S481;

SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO: 55, SEQ ID NO: 61, when one of the loci in the set is D9S930;

SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:54, when one of the loci in the set is D10S1239;

SEQ ID NO:17, SEQ ID NO:18, when one of the loci in the set is D14S118;

SEQ ID NO:19, SEQ ID NO:20, when one of the loci in the set is D14S562;

SEQ ID NO:21, SEQ ID NO:22, when one of the loci in the set is D14S548;

SEQ ID NO:23, SEQ ID NO:24, when one of the loci in the set is D16S490;

SEQ ID NO:25, SEQ ID NO:26, when one of the loci in the set is D16S753;

SEQ ID NO:27, SEQ ID NO:28, when one of the loci in the set is D17S1299;

5 SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:58, when one of the loci in the set is D16S539;

SEQ ID NO:31, SEQ ID NO:32, when one of the loci in the set is D22S683;

10 SEQ ID NO:33, SEQ ID NO:34, when one of the loci in the set is HUMCSF1PO;

SEQ ID NO:35, SEQ ID NO:36, when one of the loci in the set is HUMTPOX;

SEQ ID NO:37, SEQ ID NO:38, when one of the loci in the set is HUMTH01;

15 SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:59, SEQ ID NO:60 when one of the loci in the set is HUMvWFA31;

SEQ ID NO:41, SEQ ID NO:42, when one of the loci in the set is HUMF13A01;

20 SEQ ID NO:43, SEQ ID NO:44, when one of the loci in the set is HUMFESFPS;

SEQ ID NO:45, SEQ ID NO:46, when one of the loci in the set is HUMBFXIII;

SEQ ID NO:47, SEQ ID NO:48, when one of the loci in the set is HUMLIPOL;

25 SEQ ID NO:50, SEQ ID NO:51, when one of the loci in the set is D19S253; and

SEQ ID NO:56, SEQ ID NO:57, when one of the loci in the set is D4S2368.

30 In the method of this invention, the amplified alleles are preferably evaluated by comparing the amplified alleles to a size standard, wherein the size standard is selected from the group of size standards consisting of a

DNA marker and a locus-specific allelic ladder. The evaluation of alleles is preferably done using polyacrylamide gel electrophoresis to separate the alleles, thereby forming a polyacrylamide gel of separated alleles. The separated alleles in the polyacrylamide gel are preferably determined by
5 visualizing the alleles with an appropriate technique such as silver staining, but more preferably with fluorescent analysis.

Fluorescent analysis is preferably done by labeling one primer of each pair of primers used in the multiplex amplification reaction with a fluorescent label prior to use in the reaction. The fluorescent label used to label each
10 such primer is preferably a fluorescein label or a tetramethyl rhodamine label. Most preferably, at least two different labels are used to label the different primers which are used in the multiplex amplification reaction.

The at least one DNA sample to be analyzed using the method of this invention is preferably isolated from human tissue, preferably tissue selected
15 from the group consisting of blood, semen, vaginal cells, hair, saliva, urine, bone, buccal samples, amniotic fluid containing placental cells or fetal cells, and mixtures of any of the tissues listed above.

In an alternative embodiment, the invention is a kit for simultaneously analyzing STR sequences in at least three loci, the kit comprising a
20 container which has oligonucleotide primer pairs for co-amplifying a set of at least three short tandem repeat loci, wherein the set of loci are selected from the sets of loci consisting of:

D3S1539, D19S253, D13S317;
D10S1239, D9S930, D20S481;
25 D10S1239, D4S2368, D20S481;
D10S1239, D9S930, D4S2368;
D16S539, D7S820, D13S317;
D10S1239, D9S930, D13S317;
D3S1539, D7S820, D13S317, D5S818;
30 D17S1298, D7S820, D13S317, D5S818;
D20S481, D7S820, D13S317, D5S818;

D9S930, D7S820, D13S317, D5S818;
D10S1239, D7S820, D13S317, D5S818;
D14S118, D7S820, D13S317, D5S818;
D14S562, D7S820, D13S317, D5S818;
5 D14S548, D7S820, D13S317, D5S818;
D16S490, D7S820, D13S317, D5S818;
D17S1299, D7S820, D13S317, D5S818;
D16S539, D7S820, D13S317, D5S818;
D22S683, D7S820, D13S317, D5S818;
10 D16S753, D7S820, D13S317, D5S818;
D3S1539, D19S253, D13S317, D20S481;
D3S1539, D19S253, D4S2368, D20S481;
D10S1239, D9S930, D4S2368, D20S481;
D16S539, D7S820, D13S317, HUMvWFA31;
15 D16S539, D7S820, D13S317, D5S818, HUMCSF1PO, HUMTPOX;
D16S539, D7S820, D13S317, D5S818, HUMF13A01, HUMFESFPS;
D16S539, D7S820, D13S317, D5S818, HUMCSF1PO, HUMTPOX,
HUMTH01;
D16S539, D7S820, D13S317, D5S818, HUMF13A01, HUMFESFPS,
20 HUMBFXIII;
D16S539, D7S820, D13S317, D5S818, HUMCSF1PO, HUMTPOX,
HUMTH01, HUMvWFA31; and
D16S539, D7S820, D13S317, D5S818, HUMF13A01, HUMFESFPS,
HUMBFXIII, HUMLIPOL.

25 At least one of the primers in each primer pair included in the kit preferably has a sequence selected from one of the groups of sequences listed under the description of the method of this invention, above.

In yet a third embodiment, the invention is primer sequences and primer pairs for amplifying specific STR loci of human DNA. Use of the
30 primers and primer pairs of this invention for multiplex analysis of human DNA is demonstrated herein below. The primers of this invention are

suitable for use in the method of this invention, wherein they can be used in either labeled or unlabeled form depending, as noted above, upon how the amplified alleles are to be determined in the evaluating step of the method.

5 The present invention, in all its various embodiments described briefly above, provides a high throughput method and materials for the detection and analysis of polymorphic genetic markers using specific combinations of loci and specified conditions. By selection of the appropriate detection technique for the evaluation step, the materials and method of this invention
10 can be used in laboratories which have only a power supply and a standard apparatus for polyacrylamide gel electrophoresis or those which have the latest in equipment for fluorescent gel scanning, e.g., FluorImager™ 575 (Molecular Dynamics, Sunnyvale, CA) or the Hitachi FMBIO™ (San Bruno, CA) fluorescent scanners or the ABI 373 and ABI Prism™ 377 DNA
15 Sequencers (Applied Biosystems Division, Perkin Elmer, Foster City, CA). Thus, the method of the present invention is adaptable for a variety of uses and laboratories.

 The approach as specified in the present invention produces a savings in time, labor and materials in the analysis of loci contained within the
20 multiplexes. The method of the present invention allows three or more, even as many as eight or more, loci to be amplified together in one tube using a single amplification reaction, instead of amplifying each locus independently in separate tubes.

 The present invention has specific use in the field of forensic analysis,
25 paternity determination, monitoring of bone marrow transplantation, linkage mapping, and detection of genetic diseases and cancers. By allowing three or more loci to be amplified and analyzed simultaneously, the materials and methods of the present invention significantly increase the certainty with which one can match DNA isolated from the blood or other tissues of two
30 different individuals. The need to distinguish accurately between small amounts of tissue of different individuals is particularly acute in forensics

applications, where many convictions (and acquittals) turn on DNA typing analysis, including the analysis of STR loci.

Scientists, particularly forensic scientists, have long appreciated the need to analyze multiple polymorphic loci of DNA in order to ensure that a match between two samples of tissue is statistically significant. (Presley, L. A. et al. (1993) "The implementation of the polymerase chain reaction (PCR) HLA DQ alpha typing by the FBI laboratory," in "The Third International Symposium on Human Identification 1992," pp. 245-269; Bever, R. A., et al. (1992) "Characterization of five VNTR loci by Hae III RFLP analysis: application to paternity testing," in "The Second International Symposium on Human Identification 1991," pp. 103-128.) However, until this invention, there were few ways one could simultaneously analyze three or more STR loci in a single reaction. To realize the importance of such multiplexing capabilities, it helps to understand some of the mathematics behind DNA typing analysis.

For purposes of illustration, suppose every STR locus has a genotype (i.e., pattern of two alleles) frequency of one in ten. In other words, suppose that the chance of two randomly selected individuals have a matching type for a single STR is 1/10. However, if two different STR loci are analyzed, the chance of a random match with both systems becomes 1/100. If three STR loci are analyzed, the chances of a random match with each of the three systems become 1/1,000 and so on. Consequently, it is easy to see how increasing the number of STR loci analyzed to any number of loci over three significantly reduces the likelihood of random matches within the general population, thereby increasing the chance one can accurately identify (or eliminate) a suspect in a crime by comparing his type with crime scene evidence. Similar reasoning can be used to conclude that the method of this invention also would increase the likelihood of accurately identifying a suspected father in a paternity case, of correctly matching bone marrow tissue, of developing significant results from linkage mapping studies, and of detecting genetic diseases and cancers.

Further objects, features, and advantages of the invention will be apparent from the following detailed description of the invention and the illustrative drawings.

BRIEF DESCRIPTION OF THE DRAWING(S)

5 Figure 1 is a laser-printed image showing the fluorescent detection of the products of simultaneous amplification of the loci D3S1539, D7S820, D13S317, and D5S818 as detected with a FluorImager™ fluorescent scanner (Molecular Dynamics, Sunnyvale, CA) in Example 1.

10 Figure 2 is a laser-printed image showing the fluorescent detection of the products of simultaneous amplification of the loci D17S1298, D7S820, D13S317, and D5S818 as detected with a FluorImager™ fluorescent scanner (Molecular Dynamics, Sunnyvale, CA) in Example 2.

15 Figure 3 is a laser-printed image showing the fluorescent detection of the products of simultaneous amplification of the loci D17S1298, D7S820, D13S317, and D5S818 as detected with a FluorImager™ fluorescent scanner (Molecular Dynamics, Sunnyvale, CA) in Example 3.

20 Figure 4 is a laser-printed image showing the fluorescent detection of the products of simultaneous amplification of the loci D20S481, D7S820, D13S317, and D5S818 as detected with a FluorImager™ fluorescent scanner (Molecular Dynamics, Sunnyvale, CA) in Example 4.

25 Figure 5 is a laser-printed image showing the fluorescent detection of the products of simultaneous amplification of the loci D20S481, D7S820, D13S317, and D5S818 as detected with a FluorImager™ fluorescent scanner (Molecular Dynamics, Sunnyvale, CA) in Example 5.

30 Figure 6 is a laser-printed image showing the fluorescent detection of the products of simultaneous amplification of the loci D9S930, D7S820, D13S317, and D5S818 as detected with a FluorImager™ fluorescent scanner (Molecular Dynamics, Sunnyvale, CA) in Example 6.

30 Figure 7 is a laser-printed image showing the fluorescent detection of the products of simultaneous amplification of the loci D10S1239, D7S820,

D13S317, and D5S818 as detected with a FluorImager™ fluorescent scanner (Molecular Dynamics, Sunnyvale, CA) in Example 7.

Figure 8 is a laser-printed image showing the fluorescent detection of the products of simultaneous amplification of the loci D10S1239, D7S820, D13S317, and D5S818 as detected with a FluorImager™ fluorescent scanner (Molecular Dynamics, Sunnyvale, CA) in Example 8.

Figure 9 is a laser-printed image showing the fluorescent detection of the products of simultaneous amplification of the loci D14S118, D7S820, D13S317, and D5S818 as detected with a FluorImager™ fluorescent scanner (Molecular Dynamics, Sunnyvale, CA) in Example 9.

Figure 10 is a laser-printed image showing the fluorescent detection of the products of simultaneous amplification of the loci D14S562, D7S820, D13S317, and D5S818 as detected with a FluorImager™ fluorescent scanner (Molecular Dynamics, Sunnyvale, CA) in Example 10.

Figure 11 is a laser-printed image showing the fluorescent detection of the products of simultaneous amplification of the loci D14S548, D7S820, D13S317, and D5S818 as detected with a FluorImager™ fluorescent scanner (Molecular Dynamics, Sunnyvale, CA) in Example 11.

Figure 12 is a laser-printed image showing the fluorescent detection of the products of simultaneous amplification of the loci D16S490, D7S820, D13S317, and D5S818 as detected with a FluorImager™ fluorescent scanner (Molecular Dynamics, Sunnyvale, CA) in Example 12.

Figure 13 is a laser-printed image showing the fluorescent detection of the products of simultaneous amplification of the loci D16S753, D7S820, D13S317, and D5S818 as detected with a FluorImager™ fluorescent scanner (Molecular Dynamics, Sunnyvale, CA) in Example 13.

Figure 14 is a laser-printed image showing the fluorescent detection of the products of simultaneous amplification of the loci D17S1299, D7S820, D13S317, and D5S818 as detected with a FluorImager™ fluorescent scanner (Molecular Dynamics, Sunnyvale, CA) in Example 14.

Figure 15 is a laser-printed image showing the fluorescent detection of the products of simultaneous amplification of the loci D16S539, D7S820, D13S317, and D5S818 as detected with a FluorImager™ fluorescent scanner (Molecular Dynamics, Sunnyvale, CA) in Example 15.

5 Figure 16 is a laser-printed image showing the fluorescent detection of the products of simultaneous amplification of the loci D22S683, D7S820, D13S317, and D5S818 as detected with a FluorImager™ fluorescent scanner (Molecular Dynamics, Sunnyvale, CA) in Example 16.

10 Figure 17 is a laser-printed image showing the fluorescent detection of the products of simultaneous amplification of the loci D16S539, D7S820, D13S317, D5S818, HUMCSF1PO ("CSF1PO") and HUMTPOX ("TPOX") as detected with an FMBIO™ Fluorescent Scanner™ (Hitachi Software Engineering, San Bruno, CA) in Example 17.

15 Figure 18 is a laser-printed image showing the fluorescent detection of the products of simultaneous amplification of the loci D16S539, D7S820, D13S317, D5S818, HUMCSF1PO ("CSF1PO"), HUMTPOX ("TPOX") and HUMTH01 ("TH01") as detected with an FMBIO™ Fluorescent Scanner™ (Hitachi Software Engineering, San Bruno, CA) in Example 18.

20 Figure 19 is a laser-printed image showing the fluorescent detection of the products of simultaneous amplification of the loci D16S539, D7S820, D13S317, D5S818, HUMCSF1PO ("CSF1PO"), HUMTPOX ("TPOX"), HUMTH01 ("TH01") and HUMvWFA31 ("vWA") as detected with an FMBIO™ Fluorescent Scanner™ (Hitachi Software Engineering, San Bruno, CA) in Example 19.

25 Figure 20 is a laser-printed image showing the fluorescent detection of the products of simultaneous amplification of the loci D16S539, D7S820, D13S317, D5S818, HUMF13A01 ("F13A01") and HUMFESFPS ("FESFPS") as detected with an FMBIO™ Fluorescent Scanner™ (Hitachi Software Engineering, San Bruno, CA) in Example 20.

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Figure 21 is a laser-printed image showing the fluorescent detection of the products of simultaneous amplification of the loci D16S539, D7S820, D13S317, D5S818, HUMF13A01 ("F13A01"), HUMFESFPS ("FESFPS") and HUMBFXIII ("F13B") as detected with an FMBIO™
5 Fluorescent Scanner™ (Hitachi Software Engineering, San Bruno, CA) in Example 21.

Figure 22 is a laser-printed image showing the fluorescent detection of the products of simultaneous amplification of the loci D16S539, D7S820, D13S317, D5S818, HUMF13A01 ("F13A01"), HUMFESFPS
10 ("FESFPS"), HUMBFXIII ("F13B") and HUMLIPOL ("LPL") as detected with an FMBIO™ Fluorescent Scanner™ (Hitachi Software Engineering, San Bruno, CA) in Example 22.

Figure 23 is a laser-printed image showing the fluorescent detection of the products of simultaneous amplification of the loci D16S539, D7S820, D13S317, D5S818, HUMCSF1PO ("CSF1PO"), HUMTPOX
15 ("TPOX"), HUMTH01 ("TH01") and HUMvWFA31 ("vWA") as detected with an FMBIO™ Fluorescent Scanner™ (Hitachi Software Engineering, San Bruno, CA) in Example 23.

Figure 24 is a laser-printed image showing the fluorescent detection of the products of simultaneous amplification of the loci D3S1539, D19S253, D13S317 and D20S481 as detected with a FluorImager™
20 fluorescent scanner (Molecular Dynamics, Sunnyvale, CA) in Example 24.

Figure 25 is a laser-printed image showing the fluorescent detection of the products of simultaneous amplification of the loci D10S1239, D9S930, D4S2368 and D20S481 as detected with a FluorImager™
25 fluorescent scanner (Molecular Dynamics, Sunnyvale, CA) in Example 25.

Figure 26 is a photograph illustrating the silver stain detection of the products of simultaneous amplification of three loci, D16S539, D7S820, and D13S317, in Example 26.

Figure 27 is a photograph illustrating the silver stain detection of the products of simultaneous amplification of four loci, D16S539, D7S820, D13S317 and HUMvWFA31 ("vWA"), in Example 27.

5 Figure 28 is a photograph illustrating the silver stain detection of the products of simultaneous amplification of three loci, D10S1239, D9S930, and D13S317, in Example 28.

Figure 29 is a photograph illustrating the silver stain detection of the products of simultaneous amplification of three loci, D10S1239, D9S930, and D4S2368, in Example 29.

10 Figure 30 is a photograph illustrating the silver stain detection of the products of simultaneous amplification of four loci, D10S1239, D9S930, D4S2368 and D20S481, in Example 30.

Figure 31 is a photograph illustrating the silver stain detection of the products of simultaneous amplification of three loci, D3S1539, D19S253 and D13S317, in Example 31.

Figure 32 is a photograph illustrating the silver stain detection of the products of simultaneous amplification of four loci, D3S1539, D19S253, D4S2368 and D20S481, in Example 32.

20 Figure 33 is a photograph illustrating the silver stain detection of the products of simultaneous amplification of four loci, D3S1539, D19S253, D13S317 and D20S481, in Example 33.

Figure 34 is a photograph illustrating the silver stain detection of the products of simultaneous amplification of three loci, D10S1239, D9S930 and D20S481, in Example 34.

25 Figure 35 is a photograph illustrating the silver stain detection of the products of simultaneous amplification of three loci, D10S1239, D4S2368 and D20S481, in Example 35.

DETAILED DESCRIPTION OF THE INVENTION

A. Definitions

The following definitions are intended to assist in providing a clear and consistent understanding of the scope and detail of the terms:

5 Allelic ladder: a standard size marker consisting of amplified alleles from the locus.

Allele: a genetic variation associated with a segment of DNA, i.e., one of two or more alternate forms of a DNA sequence occupying the same locus.

10 Biochemical nomenclature: standard biochemical nomenclature is used herein in which the nucleotide bases are designated as adenine (A); thymine (T); guanine (G); and cytosine (C). Corresponding nucleotides are, for example, deoxyguanosine-5'-triphosphate (dGTP).

DNA polymorphism: the condition in which two or more different
15 nucleotide sequences in a DNA sequence coexist in the same interbreeding population.

Locus (or genetic locus): a specific position on a chromosome. Alleles of a locus are located at identical sites on homologous chromosomes.

20 Locus-specific primer: a primer that specifically hybridizes with a portion of the stated locus or its complementary strand, at least for one allele of the locus, and does not hybridize efficiently with other DNA sequences under the conditions used in the amplification method.

Polymerase chain reaction (PCR): a technique in which cycles of
25 denaturation, annealing with primer, and extension with DNA polymerase are used to amplify the number of copies of a target DNA sequence by approximately 10^6 times or more. The polymerase chain reaction process for amplifying nucleic acid is covered by U. S. Patent Nos. 4,683,195 and 4,683,202, which are incorporated herein by reference for a description of
30 the process.

Polymorphic short tandem repeat loci: STR loci in which the number of repetitive sequence elements (and net length of sequence) in a particular region of genomic DNA varies from allele to allele, and from individual to individual.

5 Polymorphism information content (PIC): a measure of the amount of polymorphism present at a locus (Botstein et al., 1980). PIC values range from 0 to 1.0, with higher values indicating greater degrees of polymorphism. This measure generally displays smaller values than the other commonly used measure, i.e., heterozygosity. For markers that are
10 highly informative (heterozygosities exceeding about 70%), the difference between heterozygosity and PIC is slight.

Primary reaction: initial reaction using the purified human genomic DNA as template for the PCR.

Primers: two single-stranded oligonucleotides or DNA fragments
15 which hybridize with opposing strands of a locus such that the 3' termini of the primers are in closest proximity.

Primer pair: two primers including, primer 1 that hybridizes to a single strand at one end of the DNA sequence to be amplified and primer 2 that hybridizes with the other end on the complementary strand of the DNA
20 sequence to be amplified.

Primer site: the area of the target DNA to which a primer hybridizes.

Secondary reaction: reamplification with the same or different primer pair using a dilution of the primary reaction as template for the PCR.

Short tandem repeat loci (STR loci): regions of the human genome
25 which contain short, repetitive sequence elements of 3 to 7 base pairs in length.

B. Selection of Multiplex Reaction Components

The method of the present invention contemplates selecting an appropriate set of loci, primers, and amplification protocols to generate
30 amplified alleles from multiple co-amplified loci which either do not overlap

in size or which are labeled in some way to make the amplified alleles which do overlap in size distinguishable from one another. In addition, this method contemplates the selection of short tandem repeat loci which are compatible for use with a single amplification protocol. The specific combinations of loci described herein are unique in this application. Combinations of loci may be rejected for either of the above two reasons, or because, in combination, one or more of the loci do not produce adequate product yield, or fragments which do not represent authentic alleles are produced in this reaction.

Successful combinations in addition to those disclosed herein can be generated by trial and error of locus combinations, by selection of primer pair sequences, and by adjustment of primer concentrations to identify an equilibrium in which all included loci may be amplified. Once the method and materials of this invention are disclosed, various methods of selecting loci, primer pairs, and amplification techniques for use in the method and kit of this invention are likely to be suggested to one skilled in the art. All such methods are intended to be within the scope of the appended claims.

Of particular importance in the practice of the method of this invention is the size range of amplified alleles produced from the individual loci which are amplified together in the multiplex amplification reaction step. For ease of analysis with current technologies, systems which can be detected by amplification of fragments smaller than 500 bases are most preferable. The most preferable combinations of loci, primers, and amplification techniques are described in the Summary of the Invention section, above.

Inappropriate selection of primers can produce several undesirable effects such as lack of amplification, amplification at multiple sites, primer dimer formation, undesirable interaction of primer sequences from different loci, production of alleles from one locus which overlap with alleles from another, or the need for amplification conditions or protocols for the different loci which are incompatible in a multiplex. Synthesis of the

primers used in the present method can be conducted using any standard procedure for oligonucleotide synthesis known to those skilled in the art.

C. Use of Multiplexes of Three Loci to Develop Multiplexes Using More than Three Loci

5 Any one of a number of different techniques can be used to select the set of STR loci to be analyzed using a method of the present invention. One preferred technique for developing useful sets of loci for use in this method of analysis is described below. Once a multiplex containing three loci is developed, it may be used as a core to create multiplexes containing
10 more than three loci. New combinations are created including the first three loci. For example, the core multiplex containing loci D7S820, D13S317, and D5S818 was used to generate derivative multiplexes of D16S539, D7S820, D13S317, and D5S818; HUMCSF1PO, HUMTPOX, D16S539, D7S820, D13S317, and D5S818; HUMCSF1PO, HUMTPOX, HUMTH01,
15 D16S539, D7S820, D13S317, and D5S818; and HUMCSF1PO, HUMTPOX, HUMTH01, HUMvWFA31, D16S539, D7S820, D13S317, and D5S818.

It is contemplated that core sets of loci can be used to generate other appropriate derivative sets of STR loci for multiplex analysis using the
20 method of this invention. Regardless of what method is used to select the loci analyzed using the method of the present invention, all the loci selected for multiplex analysis should share the following characteristics: (1) they should produce minimal slippage (e.g., from misreading the repeat sequence during an amplification step), (2) few if any artifacts due to the addition or
25 deletion of a base to the amplified alleles during the multiplex amplification step, (3) few if any artifacts due to premature termination of amplification reactions by a polymerase, and (4) no "trailing" bands of smaller molecular weight from consecutive single base deletions below a given authentic amplified allele. See, e.g., Schumm et al., "Development of Nonisotopic
30 Multiplex Amplification Sets for Analysis of Polymorphic STR Loci," *Fourth*

International Symposium on Human Identification 1993, pp. 177-187 (pub. by Promega Corp., 1993).

D. Preparation of DNA Samples

Samples of human genomic DNA can be prepared for use in the method of this invention using any method of DNA preparation which is compatible with the amplification of a single locus. Many such methods are suitable for use in preparing genomic DNA samples for use in the method of this invention, including, but not limited to, the methods of DNA sample preparation described by Patel, P. I., et al. (1984) "Organization of the HPRT gene and related sequences in the human genome," *Somat Cell Mol Genet* 10: 483-493, and Gill, P., et al. (1985) "Forensic application of DNA 'fingerprints'," *Nature* 318: 577-579.

DNA concentrations can be measured prior to use in the method of the present invention, using any standard method of DNA detection. However, the DNA concentration is preferably measured fluorometrically using a measurement technique such as that described by Brunk C. F., et al. 4 (1979) "Assay for nanogram quantities of DNA in cellular homogenates," *Anal Biochem* 92: 497-500. The DNA concentration is more preferably measured by comparison of the amount of hybridization of DNA standards with a human-specific probe such as that described by Wayne et al. (1979) Wayne, J.S., et al. (1991) "Sensitive and specific quantification of human genomic deoxyribonucleic acid (DNA) in forensic science specimens: casework examples," *J. Forensic Sci.*, 36:1198-1203. Use of too much template DNA in the amplification reactions can produce artifacts which appear as extra bands which do not represent true alleles.

E. Amplification of DNA

Once a sample of human genomic DNA is isolated, and its concentration determined as described above, the targeted loci can be co-amplified in the multiplex amplification step of the present method. Any

one of a number of different amplification methods can be used to amplify the loci, including, but not limited to, polymerase chain reaction (PCR) (Saiki, R. K., et al. (1985) "Enzymatic amplification of beta-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia," *Science* 230: 1350-1354), transcription based amplification (Kwoh, D. Y., and Kwoh, T. J. (1990) "Target amplification systems in nucleic acid-based diagnostic approaches," *American Biotechnology Laboratory*, October, 1990) and strand displacement amplification (SDA) (Walker, G. T., et al. (1992) "Isothermal in vitro amplification of DNA by a restriction enzyme-DNA Polymerase system," *Proc. Natl. Acad. Sci., U.S.A.* 89: 392-396). Preferably, the DNA sample is subjected to PCR amplification using primer pairs and thermocycling conditions specific to each locus in the set. Reference is made to the Sequence Listing at the end of this specification for details of the primer sequences used in the Examples below, some of which sequences are alternative embodiments of this invention.

Details of the most preferred amplification protocol for each of the most preferred combinations of loci for use in the method of this invention are given in the examples below. Reference is also made to the examples for additional details of the specific procedure relating to each multiplex. The sequences of the locus-specific primers used in the examples include a number of nucleotides which, under the conditions used in the hybridization, are sufficient to hybridize with an allele of the locus to be amplified and to be essentially free from amplification of alleles of other loci. Reference is made to U.S. Patent 5,192,659 to Simons, the teaching of which is incorporated herein by reference for a more detailed description of locus-specific primers.

F. Separation and Detection of DNA Fragments

Once a set of amplified alleles is produced from the multiplex amplification step of the present method, the amplified alleles are evaluated. The evaluation step of this method can be accomplished by any one of a

number of different means, the most preferred of which are described below.

Electrophoresis is preferably used to separate the products of the multiplex amplification reaction, more preferably denaturing polyacrylamide gel electrophoresis (see, e.g., Sambrook, J. et al. (1989) In *Molecular Cloning—A Laboratory Manual*, 2nd edition, Cold Spring Harbor Laboratory Press, pp. 13.45-13.57). The most preferred gel preparation and electrophoresis procedures and conditions for use in the evaluating step of the method of this invention are described in Example 1. Separation of DNA fragments in a denaturing polyacrylamide gel occurs based on fragment size.

Once the amplified alleles are separated in a polyacrylamide gel, the alleles and any other DNA in the gel (e.g., DNA markers or an allelic ladder) can then be visualized and analyzed. Visualization of the DNA in the gel can be accomplished using any one of a number of prior art techniques, including silver staining or reporters such as radioisotopes, fluorescers, chemiluminescers and enzymes in combination with detectable substrates. Silver staining is a preferred method of visualizing the alleles in the gel (see, e.g., Bassam, B. J., et al. (1991) "Fast and sensitive silver staining of DNA in polyacrylamide gels," *Anal. Biochem.* 196: 80-83). A more preferred method is the use of radioactively-labeled (see, e.g., Hammond et al., (1994)) or fluorescently-labeled (see, e.g., Schumm et al., (1994)) primers for each locus in the multiplexing reaction followed by detection of the labeled products using an autoradiogram or fluorometric detector, respectively. All three references, cited above, which describe prior art methods of visualizing alleles, are incorporated by reference herein.

The alleles present in the DNA sample are preferably determined by comparison to a size standard such as a DNA marker or a locus-specific allelic ladder to determine the alleles present at each locus within the sample. The most preferred size marker for evaluation of a multiplex amplification containing two or more polymorphic STR loci consists of a

combination of allelic ladders for each of the loci being evaluated. See, e.g., description of allelic ladders and method of ladder construction in Schumm et al., *supra*, at p. 178.

5 The preferred size marker for evaluation of a multiplex amplification containing two or more polymorphic STR loci which are generated using fluorescently-labeled primers for each locus consists of a combination of fluorescently-labeled allelic ladders for the loci being evaluated. *Id.*

10 Following the construction of allelic ladders for individual loci, they may be mixed and loaded for gel electrophoresis at the same time as the loading of amplified samples occurs. Each allelic ladder co-migrates with alleles in the sample from the corresponding locus.

15 A permanent record of the data can be generated using Automatic Processor Compatible (APC) film (STR systems manual #TMD004, available from Promega Corporation, Madison, WI) or with use of a fluorescent detection instrument (STR systems manual #TMD006, also available from Promega Corporation, Madison, WI).

G. Preferred Detection Technique: Fluorescent Detection

20 In one of the most preferred embodiments of the method of this invention, fluorescent detection is used to evaluate the amplified alleles in the mixture produced by the multiplex amplification reaction. Below is a brief summary of how that method of detection preferably is practiced.

25 With the advent of automated fluorescent imaging, faster detection and analysis of multiplex amplification products can be achieved. For fluorescent analyses, one fluoresceinated primer can be included in the amplification of each locus. Descriptions of the use of two preferred species of fluorescent labeled primers, fluorescein-labeled (FL-) and tetramethyl rhodamine-labeled (TMR-) primers are included in the examples, below. Separation of the amplified fragments produced using such labeled primers is achieved in precisely the same manner as with the silver stain
30 detection method. The resulting gel can be analyzed using a FluorImager™

analyzer (commercially available from Molecular Dynamics, Sunnyvale, CA) or FMBIO™ (commercially available from Hitachi Corporation, San Bruno, CA), which scans the gel and digitizes the data in a very short time, e.g., three to twenty minutes.

5 In summary, the method of this invention is most preferably practiced using fluorescent detection at the evaluating step. In this preferred method of detection, one of each pair of primers used in the multiplex amplification reaction has a fluorescent label attached thereto, and as a result, the amplified alleles produced from the amplification reaction are fluorescently
10 labeled. In this most preferred embodiment of the invention, the amplified alleles are subsequently separated on a polyacrylamide gel and the separated alleles visualized and analyzed using a fluorescent image analyzer.

Fluorescent detection is preferred over radioactive methods of labeling and detection, because it does not require the use of radioactive materials,
15 and all the regulatory and safety problems which accompany the use of such materials.

Fluorescent detection is also preferred over other non-radioactive methods of detection, such as silver staining, because fluorescent methods of detection generally reveal fewer gel artifacts than staining. The smaller
20 number of gel artifacts are probably due, to a large extent, to the fact that only amplified fragments of DNA with labels attached are detected in fluorescent detection, while every amplified fragment of DNA produced from the multiplex amplification reaction is stained and detected using the silver staining method of detection. Polyacrylamide gels stained with silver stain
25 also have a considerably higher general background due to nonspecific binding of silver stain to the gel itself, reducing the sensitivity with which individual bands of DNA can be detected within the gel. Silver staining and fluorescent methods of detection are compared in two sets of examples, hereinbelow.

H. Kit

The present invention is also directed to kits that utilize the process described above. A basic kit comprises a container having one or more locus-specific primers for each locus. Instructions for use optionally may be included.

Other optional kit components may include an allelic ladder directed to each of the specified loci, a sufficient quantity of enzyme for amplification, amplification buffer to facilitate the amplification, loading solution for preparation of the amplified material for gel electrophoresis, human genomic DNA as a template control, a size marker to insure that materials migrate as anticipated in the gel, and a protocol and manual to educate the user and to limit error in use. The amounts of the various reagents in the kits also can be varied depending upon a number of factors, such as the optimum sensitivity of the process. It is within the scope of this invention to provide test kits for use in manual applications or test kits for use with automated detectors or analyzers.

EXAMPLES

The following examples are presented to illustrate the advantages of the present invention and to assist one of ordinary skill in making and using the same. The examples are not intended in any way to otherwise limit the scope of the disclosure or protection granted by the patent.

Genomic DNA isolation and quantitation were performed essentially as described by Puers, C., et al. (1993) "Identification of repeat sequence heterogeneity at the polymorphic short tandem repeat locus HUMTH01 [AATG]_n and reassignment of alleles in population analysis by using a locus-specific allelic ladder," *Am. J. Hum. Genet.* 53: 953-958. These methods are generally known to those skilled in the art and are preferred, but not required, for application of the invention.

Amplification products were separated by electrophoresis through a 0.4mm thick 4% denaturing polyacrylamide gel (19:1 ratio of acrylamide to

bis-acrylamide) which contained 7 M urea (Sambrook et al., (1989)), and which was chemically cross-linked to a glass plate (Kobayashi, Y. (1988) "A method to cast thin sequencing gels," *BRL Focus* 10: 73-74) in cases involving silver stain analysis. No such cross-linking was employed in cases involving fluorescent analysis. DNA samples were mixed with 2.5 μ l of a loading solution (10mM NaOH, 95% formamide, 0.05% bromophenol blue, 0.05% xylene cyanol), denatured at 95C for 2 min., and chilled on ice prior to loading.

Once separated by polyacrylamide gel electrophoresis, the amplified reaction products and DNA size marker controls were detected using silver staining, fluorescent detection, radioactive detection, or a combination of the above detection methods. In some Examples, the reaction products and size markers in the gel were detected by silver staining using a standard method of staining and detection described in the prior art. (See, e.g., Bassam et al., (1991).) Permanent images of the stained gels were obtained by exposure to Automatic Processor Compatible Film (APC Film, Promega Corporation, Cat. No. DQ4411). In other Examples, detection was performed by fluorescent scanning, using a method described in the prior art (Schumm et al., (1994)).

Each example below is an example of the use of the method of this invention, and in some cases, an example of the use of one or more of the primers of this invention to determine simultaneously the alleles present in at least three short tandem repeat loci from one or more DNA samples. Tables 1 and 2 summarize which set of loci was co-amplified in the multiplex amplification reaction described in each Example below. The two tables also indicate which primer pair was used to amplify each such locus in each such multiplex reaction. Table 1 lists all the Examples where fluorescent scanning was used to detect the amplified alleles from the multiplex reactions described therein, while Table 2 lists the Examples where silver staining was used to detect the amplified alleles.

One primer of each primer pair listed on Table 1 was fluorescently labeled prior to being used in the multiplex amplification reaction. In some cases, a different label was used to label primers to different loci, such that the alleles produced using the different primers could be distinguished from one another when scanned by the fluorescent scanner used in the Examples below. Two different fluorescent labels were used in the Examples below, described as "FL" to indicate fluorescein-labeled and "TMR" to indicate tetramethyl rhodamine-labeled in Table 1, below. Table 1 also indicates which primer of each pair of primers used in the multiplex amplification reaction was so labeled in each example (e.g., "FL-2" means the primer with SEQ ID NO:2 was labeled at its 5' end with fluorescein prior to being used in the multiplex amplification reaction).

The same FL and TMR abbreviations are used in the Examples below. However, there the label abbreviation is placed immediately before the SEQ ID NO of the labeled primer used in the amplification reaction described therein (e.g., "FL-SEQ ID NO:2" instead of "FL-2").

In four pairs of Examples below (Examples 2 and 3, 4 and 5, 7 and 8, and 19 and 23), the same set of loci were analyzed using the same set of primers and the same fluorescent labels covalently attached to one of each pair of primers for each STR locus analyzed. However, a different set of primers was labeled in each of the Examples. These pairs of Examples are included herein to demonstrate that the same low background and identical allelic determination results can be obtained from the same set of primers using fluorescent labeling as a method of detection, no matter which of the primers of a primer pair is labeled prior to being used in a multiplex amplification reaction of the method of this invention.

TABLE 1

Example	Loci Amplified	Primer Pair: SEQ ID NO:'s	Fluorescent Label(s) Used
1	D7S820 D13S317 D5S818	1, 2 3, 4 5, 6	FL-2 FL-4 FL-6
2	D7S820 D13S317 D5S818	1, 2 3, 4 5, 6	FL-2 FL-4 FL-6
3	D7S820 D13S317 D5S818	1, 2 3, 4 5, 6	FL-2 FL-4 FL-6
4	D7S820 D13S317 D5S818	1, 2 3, 4 5, 6	FL-2 FL-4 FL-6
5	D7S820 D13S317 D5S818	1, 2 3, 4 5, 6	FL-2 FL-4 FL-6
6	D7S820 D13S317 D5S818	1, 2 3, 4 5, 6	FL-2 FL-4 FL-6
7	D7S820 D13S317 D5S818	1, 2 3, 4 5, 6	FL-2 FL-4 FL-6
8	D7S820 D13S317 D5S818	1, 2 3, 4 5, 6	FL-2 FL-4 FL-6
9	D7S820 D13S317 D5S818	1, 2 3, 4 5, 6	FL-2 FL-4 FL-6
10	D7S820 D13S317 D5S818	1, 2 3, 4 5, 6	FL-2 FL-4 FL-6
11	D7S820 D13S317 D5S818	1, 2 3, 4 5, 6	FL-2 FL-4 FL-6

12	D7S820 D13S317 D5S818	1, 2 3, 4 5, 6	FL-2 FL-4 FL-6
13	D7S820 D13S317 D5S818	1, 2 3, 4 5, 6	FL-2 FL-4 FL-6
14	D7S820 D13S317 D5S818	1, 2 3, 4 5, 6	FL-2 FL-4 FL-6
15	D7S820 D13S317 D5S818	1, 2 3, 4 5, 6	FL-2 FL-4 FL-6
16	D7S820 D13S317 D5S818	1, 2 3, 4 5, 6	FL-2 FL-4 FL-6
17	D7S820 D13S317 D5S818 D16S539 HUMCSF1	1, 2 3, 4 5, 6 29, 30 33, 34	FL-2 FL-4 FL-6 FL-30 TMR-33
18	D7S820 D13S317 D5S818 D16S539 HUMCSF1 PO	1, 2 3, 4 5, 6 29, 30 33, 34 35, 36	FL-2 FL-4 FL-6 FL-30 TMR-33 TMR-36
19	D7S820 D13S317 D5S818 D16S539 HUMCSF1 PO	1, 2 3, 4 5, 6 29, 30 33, 34 35, 36	FL-2 FL-4 FL-6 FL-30 TMR-33 TMR-36
20	D7S820 D13S317 D5S818 D16S539 HUMF13A	1, 2 3, 4 5, 6 29, 30 41, 42	FL-2 FL-4 FL-6 FL-30 TMR-41

21	D7S820 D13S317 D5S818 D16S539 HUMF13A 01	1, 2 3, 4 5, 6 29, 30 41, 42 43, 44	FL-2 FL-4 FL-6 FL-30 TMR-41 TMR-43
22	D7S820 D13S317 D5S818 D16S539 HUMF13A 01	1, 2 3, 4 5, 6 29, 30 41, 42 43, 44	FL-2 FL-4 FL-6 FL-30 TMR-41 TMR-43
23	D7S820 D13S317 D5S818	1, 2 3, 4 5, 6	TMR-2 TMR-4 TMR-6
24	D3S1539 D19S253 D13S317	7, 49 50, 51 3, 4	FL-49 FL-50 FL-4
25	D10S1239 D9S930 D4S2368	15, 54 55, 14 56, 57	FL-15 FL-14 FL-57

5

Note that in a few cases, the same set of loci and same set of primer pairs appear in Table 1 and in Table 2. In such cases, the same set of alleles were analyzed using fluorescent detection and silver staining, respectively. Two such cases of duplicate sets are provided herein, Examples 24 and 33, and Examples 25 and 30. These examples clearly illustrate that the same results can be obtained with either method.

10

TABLE 2:

Example	Loci Amplified	Primer Pair: SEQ ID NO:'s
26	D16S539 D7S820	29, 58 1, 2

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27	D16S539 D7S820 D13S317	29, 30 1, 2 3, 4
28	D10S1239 D9S930	15, 54 55, 61
29	D10S1239 D9S930	15, 54 55, 61
30	D10S1239 D9S930 D4S2368	15, 54 55, 14 56, 57
31	D3S1539 D19S253	7, 49 50, 51
32	D3S1539 D19S253 D4S2368	7, 49 50, 51 56, 57
33	D3S1539 D19S253 D13S317	7, 49 50, 51 3, 4
34	D10S1239 D9S930	15, 54 55, 14
35	D10S1239 D4S2368	15, 54 56, 57

EXAMPLE 1

Fluorescent Detection of Multiplex Amplification of
Loci D3S1539, D7S820, D13S317, and D5S818

In this example, a DNA template was amplified simultaneously at the individual loci D3S1539, D7S820, D13S317, and D5S818 in a single reaction vessel. The PCR amplification was performed in 25 μ l of 1x STR Buffer (50mM KCl, 10mM Tris-HCl (pH 9.0 at 25°C), 0.1% Triton X-100, 1.5mM MgCl₂ and 200 μ M each of dATP, dCTP, dGTP and dTTP) using 5 ng template, and 0.04 U *Taq* DNA Polymerase/ μ l. A Thermal Cycler 480

(Perkin Elmer, Foster City, CA) was employed with the following amplification protocol: 96°C for 2 min., then 10 cycles of 94°C for 1 min., 60°C for 1 min., and 70°C for 1.5 min., followed by 20 cycles of 90°C for 1 min., 60°C for 1 min., 70°C for 1.5 min., followed by 1 cycle of 60°C for 30 min.

Eight amplification primers were used in combination, including .25 μ M each D3S1539 primers 1 (SEQ ID NO:7) and 2 (FL-SEQ ID NO:8), .325 μ M each D7S820 primers 1 (SEQ ID NO:1) and 2 (FL-SEQ ID NO:2), .219 μ M each D13S317 primers 1 (SEQ ID NO:3) and 2 (FL-SEQ ID NO:4), .375 μ M each D5S818 primers 1 (SEQ ID NO:5) and 2 (FL-SEQ ID NO:6).

Amplified products were separated by denaturing polyacrylamide gel electrophoresis on a 32 cm gel for 45 min. at 40 W and products were visualized by detection of the fluorescent signals using the FluorImager™ fluorescent scanner (Molecular Dynamics, Sunnyvale, CA).

Reference is made to Figure 1 which displays the amplified fragments of each locus. Lanes 1 to 5 contain DNA samples simultaneously co-amplified for the loci D3S1539, D7S820, D13S317, and D5S818.

EXAMPLE 2

Fluorescent Detection of Multiplex Amplification of Loci D17S1298, D7S820, D13S317, and D5S818

In this example, a DNA template was amplified simultaneously at the individual loci D17S1298, D7S820, D13S317, and D5S818 in a single reaction vessel. The PCR amplification was performed in 25 μ l of 1x STR Buffer (50mM KCl, 10mM Tris-HCl (pH 9.0 at 25°C), 0.1% Triton X-100, 1.5mM MgCl₂ and 200 μ M each of dATP, dCTP, dGTP and dTTP) using 5 ng template, and 0.04 U *Taq* DNA Polymerase/ μ l. A Thermal Cycler 480 (Perkin Elmer, Foster City, CA) was employed with the following amplification protocol: 96°C for 2 min., then 10 cycles of 94°C for 1 min., 60°C for 1 min., and 70°C for 1.5 min., followed by 20 cycles of 90°C for

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1 min., 60°C for 1 min., 70°C for 1.5 min., followed by 1 cycle of 60°C for 30 min.

Eight amplification primers were used in combination, including .25 μ M each D17S1298 primers 1 [SEQ ID NO:9] and 2 [FL-SEQ ID NO:10],
5 .325 μ M each D7S820 primers 1 [SEQ ID NO:1] and 2 [FL-SEQ ID NO:2],
.219 μ M each D13S317 primers 1 [SEQ ID NO:3] and 2 [FL-SEQ ID NO:4],
.375 μ M each D5S818 primers 1 [SEQ ID NO:5] and 2 [FL-SEQ ID NO:6].

Amplified products were separated by denaturing polyacrylamide gel electrophoresis on a 32 cm gel for 45 min. at 40 W and products were
10 visualized by detection of the fluorescent signals using the FluorImager™ fluorescent scanner (Molecular Dynamics, Sunnyvale, CA).

Reference is made to Figure 2 which displays the amplified fragments of each locus. Lanes 1 to 5 contain DNA samples simultaneously co-amplified for the loci D17S1298, D7S820, D13S317, and D5S818.

15

EXAMPLE 3

Fluorescent Detection of Multiplex Amplification of Loci D17S1298, D7S820, D13S317, and D5S818

The loci D17S1298, D7S820, D13S317, and D5S818 were amplified as described in Example 2 except that SEQ ID NO:9 was replaced with FL-
20 SEQ ID NO:9 and FL-SEQ ID NO:10 was replaced with SEQ ID NO:10.

Amplified products were separated by denaturing polyacrylamide gel electrophoresis on a 32 cm gel for 45 min. at 40 W and products were visualized by detection of the fluorescent signals using the FluorImager™ fluorescent scanner (Molecular Dynamics, Sunnyvale, CA).

25 Reference is made to Figure 3 which displays the amplified fragments of each locus. Lanes 1 to 5 contain DNA samples simultaneously co-amplified for the loci D17S1298, D7S820, D13S317, and D5S818.

EXAMPLE 4

Fluorescent Detection of Multiplex Amplification of Loci D20S481, D7S820, D13S317, and D5S818

In this example, a DNA template was amplified simultaneously at the individual loci D20S481, D7S820, D13S317, and D5S818 in a single reaction vessel. The PCR amplification was performed in 25 μ l of 1x STR Buffer (50mM KCl, 10mM Tris-HCl (pH 9.0 at 25°C), 0.1% Triton X-100, 1.5mM MgCl₂ and 200 μ M each of dATP, dCTP, dGTP and dTTP) using 5 ng template, and 0.04 U *Taq* DNA Polymerase/ μ l. A Thermal Cycler 480 (Perkin Elmer, Foster City, CA) was employed with the following amplification protocol: 96°C for 2 min., then 10 cycles of 94°C for 1 min., 60°C for 1 min., and 70°C for 1.5 min., followed by 20 cycles of 90°C for 1 min., 60°C for 1 min., 70°C for 1.5 min., followed by 1 cycle of 60°C for 30 min.

Eight amplification primers were used in combination, including .25 μ M each D20S481 primers 1 [SEQ ID NO:11] and 2 [FL-SEQ ID NO:12], .325 μ M each D7S820 primers 1 [SEQ ID NO:1] and 2 [FL-SEQ ID NO:2], .219 μ M each D13S317 primers 1 [SEQ ID NO:3] and 2 [FL-SEQ ID NO:4], .375 μ M each D5S818 primers 1 [SEQ ID NO:5] and 2 [FL-SEQ ID NO:6].

Amplified products were separated by denaturing polyacrylamide gel electrophoresis on a 32 cm gel for 45 min. at 40 W and products were visualized by detection of the fluorescent signals using the FluorImager™ fluorescent scanner (Molecular Dynamics, Sunnyvale, CA).

Reference is made to Figure 4 which displays the amplified fragments of each locus. Lanes 1 to 5 contain DNA samples simultaneously co-amplified for the loci D20S481, D7S820, D13S317, and D5S818.

EXAMPLE 5

Fluorescent Detection of Multiplex Amplification of Loci D20S481, D7S820, D13S317, and D5S818

The loci D20S481, D7S820, D13S317, and D5S818 were amplified
5 as described in Example 4 except that SEQ ID NO:11 was replaced with FL-
SEQ ID NO:11 and FL-SEQ ID NO:12 was replaced with SEQ ID NO:12.

Amplified products were separated by denaturing polyacrylamide gel
electrophoresis on a 32 cm gel for 45 min. at 40 W and products were
visualized by detection of the fluorescent signals using the FluorImager™
10 fluorescent scanner (Molecular Dynamics, Sunnyvale, CA).

Reference is made to Figure 5 which displays the amplified fragments
of each locus. Lanes 1 to 5 contain DNA samples simultaneously co-
amplified for the loci D20S481, D7S820, D13S317, and D5S818.

EXAMPLE 6

15 Fluorescent Detection of Multiplex Amplification of Loci D9S930, D7S820, D13S317, and D5S818

In this example, a DNA template was amplified simultaneously at the
individual loci D9S930, D7S820, D13S317, and D5S818 in a single
reaction vessel. The PCR amplification was performed in 25 μ l of 1x STR
20 Buffer (50mM KCl, 10mM Tris-HCl (pH 9.0 at 25°C), 0.1% Triton X-100,
1.5mM MgCl₂ and 200 μ M each of dATP, dCTP, dGTP and dTTP) using 5
ng template, and 0.04 U *Taq* DNA Polymerase/ μ l. A Thermal Cycler 480
(Perkin Elmer, Foster City, CA) was employed with the following
amplification protocol: 96°C for 2 min., then 10 cycles of 94°C for 1 min.,
25 60°C for 1 min., and 70°C for 1.5 min., followed by 20 cycles of 90°C for
1 min., 60°C for 1 min., 70°C for 1.5 min., followed by 1 cycle of 60°C for
30 min.

Eight amplification primers were used in combination, including .70
 μ M each D9S930 primers 1 [SEQ ID NO:13] and 2 [FL-SEQ ID NO:14], .325

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μ M each D7S820 primers 1 [SEQ ID NO:1] and 2 [FL-SEQ ID NO:2], .22 μ M each D13S317 primers 1 [SEQ ID NO:3] and 2 [FL-SEQ ID NO:4], .375 μ M each D5S818 primers 1 [SEQ ID NO:5] and 2 [FL-SEQ ID NO:6].

Amplified products were separated by denaturing polyacrylamide gel electrophoresis on a 32 cm gel for 45 min. at 40 W and products were visualized by detection of the fluorescent signals using the FluorImagerTM fluorescent scanner (Molecular Dynamics, Sunnyvale, CA).

Reference is made to Figure 6 which displays the amplified fragments of each locus. Lanes 1 to 5 contain DNA samples simultaneously co-amplified for the loci D9S930, D7S820, D13S317, and D5S818.

EXAMPLE 7

Fluorescent Detection of Multiplex Amplification of Loci D10S1239, D7S820, D13S317, and D5S818

In this example, a DNA template was amplified simultaneously at the individual loci D10S1239, D7S820, D13S317, and D5S818 in a single reaction vessel. The PCR amplification was performed in 25 μ l of 1x STR Buffer (50mM KCl, 10mM Tris-HCl (pH 9.0 at 25°C), 0.1% Triton X-100, 1.5mM MgCl₂ and 200 μ M each of dATP, dCTP, dGTP and dTTP) using 5 ng template, and 0.04 U *Taq* DNA Polymerase/ μ l. A Thermal Cycler 480 (Perkin Elmer, Foster City, CA) was employed with the following amplification protocol: 96°C for 2 min., then 10 cycles of 94°C for 1 min., 60°C for 1 min., and 70°C for 1.5 min., followed by 20 cycles of 90°C for 1 min., 60°C for 1 min., 70°C for 1.5 min., followed by 1 cycle of 60°C for 30 min.

Eight amplification primers were used in combination, including .75 μ M each D10S1239 primers 1 [SEQ ID NO:15] and 2 [FL-SEQ ID NO:16], .325 μ M each D7S820 primers 1 [SEQ ID NO:1] and 2 [FL-SEQ ID NO:2], .22 μ M each D13S317 primers 1 [SEQ ID NO:3] and 2 [FL-SEQ ID NO:4], .375 μ M each D5S818 primers 1 [SEQ ID NO:5] and 2 [FL-SEQ ID NO:6].

Amplified products were separated by denaturing polyacrylamide gel electrophoresis on a 32 cm gel for 45 min. at 40 W and products were visualized by detection of the fluorescent signals using the FluorImager™ fluorescent scanner (Molecular Dynamics, Sunnyvale, CA).

5 Reference is made to Figure 7 which displays the amplified fragments of each locus. Lanes 1 to 5 contain DNA samples simultaneously co-amplified for the loci D10S1239, D7S820, D13S317, and D5S818.

EXAMPLE 8

10 Fluorescent Detection of Multiplex Amplification of
Loci D10S1239, D7S820, D13S317, and D5S818

The loci D10S1239, D7S820, D13S317, and D5S818 were amplified as described in Example 7 except that SEQ ID NO:15 was replaced with FL-SEQ ID NO:15 and FL-SEQ ID NO:16 was replaced with SEQ ID NO:16.

15 Amplified products were separated by denaturing polyacrylamide gel electrophoresis on a 32 cm gel for 45 min. at 40 W and products were visualized by detection of the fluorescent signals using the FluorImager™ fluorescent scanner (Molecular Dynamics, Sunnyvale, CA).

Reference is made to Figure 8 which displays the amplified fragments of each locus. Lanes 1 to 5 contain DNA samples simultaneously co-
20 amplified for the loci D10S1239, D7S820, D13S317, and D5S818.

EXAMPLE 9

Fluorescent Detection of Multiplex Amplification of
Loci D14S118, D7S820, D13S317, and D5S818

25 In this example, a DNA template was amplified simultaneously at the individual loci D14S118, D7S820, D13S317, and D5S818 in a single reaction vessel. The PCR amplification was performed in 25 μ l of 1x STR Buffer (50mM KCl, 10mM Tris-HCl (pH 9.0 at 25°C), 0.1% Triton X-100, 1.5mM MgCl₂ and 200 μ M each of dATP, dCTP, dGTP and dTTP) using 5

ng template, and 0.04 U *Taq* DNA Polymerase/ μ l. A Thermal Cycler 480 (Perkin Elmer, Foster City, CA) was employed with the following amplification protocol: 96°C for 2 min., then 10 cycles of 94°C for 1 min., 60°C for 1 min., and 70°C for 1.5 min., followed by 20 cycles of 90°C for 1 min., 60°C for 1 min., 70°C for 1.5 min., followed by 1 cycle of 60°C for 30 min.

Eight amplification primers were used in combination, including .50 μ M each D14S118 primers 1 [SEQ ID NO:17] and 2 [FL-SEQ ID NO:18], .325 μ M each D7S820 primers 1 [SEQ ID NO:1] and 2 [FL-SEQ ID NO:2], .22 μ M each D13S317 primers 1 [SEQ ID NO:3] and 2 [FL-SEQ ID NO:4], .375 μ M each D5S818 primers 1 [SEQ ID NO:5] and 2 [FL-SEQ ID NO:6].

Amplified products were separated by denaturing polyacrylamide gel electrophoresis on a 32 cm gel for 45 min. at 40 W and products were visualized by detection of the fluorescent signals using the FluorImager™ fluorescent scanner (Molecular Dynamics, Sunnyvale, CA).

Reference is made to Figure 9 which displays the amplified fragments of each locus. Lanes 1 to 5 contain DNA samples simultaneously co-amplified for the loci D14S118, D7S820, D13S317, and D5S818.

EXAMPLE 10

Fluorescent Detection of Multiplex Amplification of Loci D14S562, D7S820, D13S317, and D5S818

In this example, a DNA template was amplified simultaneously at the individual loci D14S562, D7S820, D13S317, and D5S818 in a single reaction vessel. The PCR amplification was performed in 25 μ l of 1x STR Buffer (50mM KCl, 10mM Tris-HCl (pH 9.0 at 25°C), 0.1% Triton X-100, 1.5mM MgCl₂ and 200 μ M each of dATP, dCTP, dGTP and dTTP) using 5 ng template, and 0.04 U *Taq* DNA Polymerase/ μ l. A Thermal Cycler 480 (Perkin Elmer, Foster City, CA) was employed with the following amplification protocol: 96°C for 2 min., then 10 cycles of 94°C for 1 min., 60°C for 1 min., and 70°C for 1.5 min., followed by 20 cycles of 90°C for

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1 min., 60°C for 1 min., 70°C for 1.5 min., followed by 1 cycle of 60°C for 30 min.

Eight amplification primers were used in combination, including .50 μ M each D14S562 primers 1 [FL-SEQ ID NO:19] and 2 [SEQ ID NO:20],
5 .325 μ M each D7S820 primers 1 [SEQ ID NO:1] and 2 [FL-SEQ ID NO:2],
.22 μ M each D13S317 primers 1 [SEQ ID NO:3] and 2 [FL-SEQ ID NO:4],
.375 μ M each D5S818 primers 1 [SEQ ID NO:5] and 2 [FL-SEQ ID NO:6].

Amplified products were separated by denaturing polyacrylamide gel electrophoresis on a 32 cm gel for 45 min. at 40 W and products were
10 visualized by detection of the fluorescent signals using the FluorImager™ fluorescent scanner (Molecular Dynamics, Sunnyvale, CA).

Reference is made to Figure 10 which displays the amplified fragments of each locus. Lanes 1 to 5 contain DNA samples simultaneously co-amplified for the loci D14S562, D7S820, D13S317, and D5S818.

15

EXAMPLE 11

Fluorescent Detection of Multiplex Amplification of Loci D14S548, D7S820, D13S317, and D5S818

In this example, a DNA template was amplified simultaneously at the individual loci D14S548, D7S820, D13S317, and D5S818 in a single
20 reaction vessel. The PCR amplification was performed in 25 μ l of 1x STR Buffer (50mM KCl, 10mM Tris-HCl (pH 9.0 at 25°C), 0.1% Triton X-100, 1.5mM MgCl₂ and 200 μ M each of dATP, dCTP, dGTP and dTTP) using 5 ng template, and 0.04 U *Taq* DNA Polymerase/ μ l. A Thermal Cycler 480 (Perkin Elmer, Foster City, CA) was employed with the following
25 amplification protocol: 96°C for 2 min., then 10 cycles of 94°C for 1 min., 60°C for 1 min., and 70°C for 1.5 min., followed by 20 cycles of 90°C for 1 min., 60°C for 1 min., 70°C for 1.5 min., followed by 1 cycle of 60°C for 30 min.

Eight amplification primers were used in combination, including .50
30 μ M each D14S548 primers 1 [SEQ ID NO:21] and 2 [FL-SEQ ID NO:22].

.325 μ M each D7S820 primers 1 [SEQ ID NO:1] and 2 [FL-SEQ ID NO:2],
.22 μ M each D13S317 primers 1 [SEQ ID NO:3] and 2 [FL-SEQ ID NO:4],
.375 μ M each D5S818 primers 1 [SEQ ID NO:5] and 2 [FL-SEQ ID NO:6].

Amplified products were separated by denaturing polyacrylamide gel
5 electrophoresis on a 32 cm gel for 45 min. at 40 W and products were
visualized by detection of the fluorescent signals using the FluorImager™
fluorescent scanner (Molecular Dynamics, Sunnyvale, CA).

Reference is made to Figure 11 which displays the amplified
fragments of each locus. Lanes 1 to 5 contain DNA samples simultaneously
10 co-amplified for the loci D14S548, D7S820, D13S317, and D5S818.

EXAMPLE 12

Fluorescent Detection of Multiplex Amplification of Loci D16S490, D7S820, D13S317, and D5S818

In this example, a DNA template was amplified simultaneously at the
15 individual loci D16S490, D7S820, D13S317, and D5S818 in a single
reaction vessel. The PCR amplification was performed in 25 μ l of 1x STR
Buffer (50mM KCl, 10mM Tris-HCl (pH 9.0 at 25°C), 0.1% Triton X-100,
1.5mM MgCl₂ and 200 μ M each of dATP, dCTP, dGTP and dTTP) using 5
ng template, and 0.04 U *Taq* DNA Polymerase/ μ l. A Thermal Cycler 480
20 (Perkin Elmer, Foster City, CA) was employed with the following
amplification protocol: 96°C for 2 min., then 10 cycles of 94°C for 1 min.,
60°C for 1 min., and 70°C for 1.5 min., followed by 20 cycles of 90°C for
1 min., 60°C for 1 min., 70°C for 1.5 min., followed by 1 cycle of 60°C for
30 min.

25 Eight amplification primers were used in combination, including .50
 μ M each D16S490 primers 1 [FL-SEQ ID NO:23] and 2 [SEQ ID NO:24],
.325 μ M each D7S820 primers 1 [SEQ ID NO:1] and 2 [FL-SEQ ID NO:2],
.22 μ M each D13S317 primers 1 [SEQ ID NO:3] and 2 [FL-SEQ ID NO:4],
.375 μ M each D5S818 primers 1 [SEQ ID NO:5] and 2 [FL-SEQ ID NO:6].

Amplified products were separated by denaturing polyacrylamide gel electrophoresis on a 32 cm gel for 45 min. at 40 W and products were visualized by detection of the fluorescent signals using the FluorImager™ fluorescent scanner (Molecular Dynamics, Sunnyvale, CA).

5 Reference is made to Figure 12 which displays the amplified fragments of each locus. Lanes 1 to 5 contain DNA samples simultaneously co-amplified for the loci D16S490, D7S820, D13S317, and D5S818.

EXAMPLE 13

10 Fluorescent Detection of Multiplex Amplification of Loci D16S753, D7S820, D13S317, and D5S818

In this example, a DNA template was amplified simultaneously at the individual loci D16S753, D7S820, D13S317, and D5S818 in a single reaction vessel. The PCR amplification was performed in 25 μ l of 1x STR Buffer (50mM KCl, 10mM Tris-HCl (pH 9.0 at 25°C), 0.1% Triton X-100, 15 1.5mM MgCl₂ and 200 μ M each of dATP, dCTP, dGTP and dTTP) using 5 ng template, and 0.04 U *Taq* DNA Polymerase/ μ l. A Thermal Cycler 480 (Perkin Elmer, Foster City, CA) was employed with the following amplification protocol: 96°C for 2 min., then 10 cycles of 94°C for 1 min., 60°C for 1 min., and 70°C for 1.5 min., followed by 20 cycles of 90°C for 20 1 min., 60°C for 1 min., 70°C for 1.5 min., followed by 1 cycle of 60°C for 30 min.

Eight amplification primers were used in combination, including .50 μ M each D16S753 primers 1 [SEQ ID NO:25] and 2 [FL-SEQ ID NO:26], .325 μ M each D7S820 primers 1 [SEQ ID NO:1] and 2 [FL-SEQ ID NO:2], 25 .22 μ M each D13S317 primers 1 [SEQ ID NO:3] and 2 [FL-SEQ ID NO:4], .375 μ M each D5S818 primers 1 [SEQ ID NO:5] and 2 [FL-SEQ ID NO:6].

Reference is made to Figure 13 which displays the amplified fragments of each locus. Lanes 1 to 5 contain DNA samples simultaneously co-amplified for the loci D16S753, D7S820, D13S317, and D5S818.

EXAMPLE 14

5 Fluorescent Detection of Multiplex Amplification of
 Loci D17S1299, D7S820, D13S317, and D5S818

In this example, a DNA template was amplified simultaneously at the individual loci D17S1299, D7S820, D13S317, and D5S818 in a single reaction vessel. The PCR amplification was performed in 25 μ l of 1x STR
10 Buffer (50mM KCl, 10mM Tris-HCl (pH 9.0 at 25°C), 0.1% Triton X-100, 1.5mM MgCl₂ and 200 μ M each of dATP, dCTP, dGTP and dTTP) using 5 ng template, and 0.04 U *Taq* DNA Polymerase/ μ l. A Thermal Cycler 480 (Perkin Elmer, Foster City, CA) was employed with the following amplification protocol: 96°C for 2 min., then 10 cycles of 94°C for 1 min.,
15 60°C for 1 min., and 70°C for 1.5 min., followed by 20 cycles of 90°C for 1 min., 60°C for 1 min., 70°C for 1.5 min., followed by 1 cycle of 60°C for 30 min.

Eight amplification primers were used in combination, including .50 μ M each D17S1299 primers 1 [SEQ ID NO:27] and 2 [FL-SEQ ID NO:28],
20 .325 μ M each D7S820 primers 1 [SEQ ID NO:1] and 2 [FL-SEQ ID NO:2], .22 μ M each D13S317 primers 1 [SEQ ID NO:3] and 2 [FL-SEQ ID NO:4], .375 μ M each D5S818 primers 1 [SEQ ID NO:5] and 2 [FL-SEQ ID NO:6].

Amplified products were separated by denaturing polyacrylamide gel electrophoresis on a 32 cm gel for 45 min. at 40 W and products were
25 visualized by detection of the fluorescent signals using the FluorImager™ fluorescent scanner (Molecular Dynamics, Sunnyvale, CA).

Reference is made to Figure 14 which displays the amplified fragments of each locus. Lanes 1 to 5 contain DNA samples simultaneously co-amplified for the loci D17S1299, D7S820, D13S317, and D5S818.

EXAMPLE 15

Fluorescent Detection of Multiplex Amplification of Loci D16S539, D7S820, D13S317, and D5S818

In this example, a DNA template was amplified simultaneously at the individual loci D16S539, D7S820, D13S317, and D5S818 in a single reaction vessel. The PCR amplification was performed in 25 μ l of 1x STR Buffer (50mM KCl, 10mM Tris-HCl (pH 9.0 at 25°C), 0.1% Triton X-100, 1.5mM MgCl₂ and 200 μ M each of dATP, dCTP, dGTP and dTTP) using 5 ng template, and 0.04 U *Taq* DNA Polymerase/ μ l. A Thermal Cycler 480 (Perkin Elmer, Foster City, CA) was employed with the following amplification protocol: 96°C for 2 min., then 10 cycles of 94°C for 1 min., 60°C for 1 min., and 70°C for 1.5 min., followed by 20 cycles of 90°C for 1 min., 60°C for 1 min., 70°C for 1.5 min., followed by 1 cycle of 60°C for 30 min.

Eight amplification primers were used in combination, including .60 μ M each D16S539 primers 1 (SEQ ID NO:29) and 2 (FL-SEQ ID NO:30), .325 μ M each D7S820 primers 1 (SEQ ID NO:1) and 2 (FL-SEQ ID NO:2), .22 μ M each D13S317 primers 1 (SEQ ID NO:3) and 2 (FL-SEQ ID NO:4), .50 μ M each D5S818 primers 1 (SEQ ID NO:5) and 2 (FL-SEQ ID NO:6).

Amplified products were separated by denaturing polyacrylamide gel electrophoresis on a 32 cm gel for 50 min. at 40 W and products were visualized by detection of the fluorescent signals using the FluorImager™ fluorescent scanner (Molecular Dynamics, Sunnyvale, CA).

Reference is made to Figure 15 which displays the amplified fragments of each locus. Lanes 1 to 5 contain DNA samples simultaneously co-amplified for the loci D16S539, D7S820, D13S317, and D5S818.

EXAMPLE 16

Fluorescent Detection of Multiplex Amplification of Loci D22S683, D7S820, D13S317, and D5S818

In this example, a DNA template was amplified simultaneously at the individual loci D22S683, D7S820, D13S317, and D5S818 in a single reaction vessel. The PCR amplification was performed in 25 μ l of 1x STR Buffer (50mM KCl, 10mM Tris-HCl (pH 9.0 at 25°C), 0.1% Triton X-100, 1.5mM MgCl₂ and 200 μ M each of dATP, dCTP, dGTP and dTTP) using 5 ng template, and 0.04 U *Taq* DNA Polymerase/ μ l. A Thermal Cycler 480 (Perkin Elmer, Foster City, CA) was employed with the following amplification protocol: 96°C for 2 min., then 10 cycles of 94°C for 1 min., 60°C for 1 min., and 70°C for 1.5 min., followed by 20 cycles of 90°C for 1 min., 60°C for 1 min., 70°C for 1.5 min., followed by 1 cycle of 60°C for 30 min.

Eight amplification primers were used in combination, including .50 μ M each D22S683 primers 1 [SEQ ID NO:31] and 2 [FL-SEQ ID NO:32], .325 μ M each D7S820 primers 1 [SEQ ID NO:1] and 2 [FL-SEQ ID NO:2], .22 μ M each D13S317 primers 1 [SEQ ID NO:3] and 2 [FL-SEQ ID NO:4], .375 μ M each D5S818 primers 1 [SEQ ID NO:5] and 2 [FL-SEQ ID NO:6].

Amplified products were separated by denaturing polyacrylamide gel electrophoresis on a 32 cm gel for 55 min. at 40 W and products were visualized by detection of the fluorescent signals using the FluorImager™ fluorescent scanner (Molecular Dynamics, Sunnyvale, CA).

Reference is made to Figure 16 which displays the amplified fragments of each locus. Lanes 1 to 5 contain DNA samples simultaneously co-amplified for the loci D22S683, D7S820, D13S317, and D5S818.

EXAMPLE 17

Fluorescent Detection of Multiplex Amplification of
Loci D16S539, D7S820, D13S317, D5S818,
HUMCSF1PO and HUMTPOX

5 In this example, a DNA template was amplified simultaneously at the individual loci D16S539, D7S820, D13S317, D5S818, HUMCSF1PO and HUMTPOX in a single reaction vessel. The PCR-amplification was performed in 25 μ l of 1x STR Buffer (50mM KCl, 10mM Tris-HCl (pH 9.0 at 25°C), 0.1% Triton X-100, 1.5mM MgCl₂ and 200 μ M each of dATP,
10 dCTP, dGTP and dTTP) using 5 ng template, and 0.06 U *Taq* DNA Polymerase/ μ l. A Thermal Cycler 480 (Perkin Elmer, Foster City, CA) was employed with the following amplification protocol: 96°C for 2 min., then 10 cycles of 94°C for 1 min., 60°C for 1 min., and 70°C for 1.5 min., followed by 20 cycles of 90°C for 1 min., 60°C for 1 min., 70°C for
15 1.5 min., followed by 1 cycle of 60°C for 30 min.

 Twelve amplification primers were used in combination, including .65 μ M each D16S539 primers 1 [SEQ ID NO:29] and 2 [FL-SEQ ID NO:30], .325 μ M each D7S820 primers 1 [SEQ ID NO:1] and 2 [FL-SEQ ID NO:2], .22 μ M each D13S317 primers 1 [SEQ. ID.3] and 2 [FL-SEQ ID NO:4], .55
20 μ M each D5S818 primers 1 [SEQ ID NO:5] and 2 [FL-SEQ ID NO:6], .40 μ M each HUMCSF1PO primers 1 [TMR-SEQ ID NO:33] and 2 [SEQ ID NO:34], .40 μ M each HUMTPOX primers 1 [SEQ ID NO:35] and 2 [TMR-SEQ ID NO:36].

 Amplified products were separated by denaturing polyacrylamide gel
25 electrophoresis on a 32 cm gel for 50 min. at 40 W and products were visualized by detection of the fluorescent signals using the FMBIOFluorImager™ (Hitachi Software Engineering, San Bruno, CA).

 Reference is made to Figure 17 which displays the amplified fragments of each locus in separate 505 nm and 625 nm scans of the same gel revealing fluorescein-labeled and tetramethyl-rhodamine labeled material,
30 respectively. Lanes 1 to 4 contain DNA samples simultaneously co-

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amplified for the loci D16S539, D7S820, D13S317, D5S818, HUMCSF1PO ("CSF1PO") and HUMTPOX ("TPOX").

EXAMPLE 18

5 Fluorescent Detection of Multiplex Amplification of
 Loci D16S539, D7S820, D13S317, D5S818, HUMCSF1PO,
 HUMTPOX and HUMTH01

In this example, a DNA template was amplified simultaneously at the individual loci D16S539, D7S820, D13S317, D5S818, HUMCSF1PO, HUMTPOX and HUMTH01 in a single reaction vessel. The PCR
10 amplification was performed in 25 μ l of 1x STR Buffer (50mM KCl, 10mM Tris-HCl (pH 9.0 at 25°C), 0.1% Triton X-100, 1.5mM MgCl₂ and 200 μ M each of dATP, dCTP, dGTP and dTTP) using 5 ng template, and 0.07 U *Taq* DNA Polymerase/ μ l. A Thermal Cycler 480 (Perkin Elmer, Foster City, CA) was employed with the following amplification protocol: 96°C for 2 min.,
15 then 10 cycles of 94°C for 1 min., 60°C for 1 min., and 70°C for 1.5 min., followed by 20 cycles of 90°C for 1 min., 60°C for 1 min., 70°C for 1.5 min., followed by 1 cycle of 60°C for 30 min.

Fourteen amplification primers were used in combination, including .75 μ M each D16S539 primers 1 [SEQ ID NO:29] and 2 [FL-SEQ ID NO:30],
20 .40 μ M each D7S820 primers 1 [SEQ ID NO:1] and 2 [FL-SEQ ID NO:2], .30 μ M each D13S317 primers 1 [SEQ ID NO:3] and 2 [FL-SEQ ID NO:4], .60 μ M each D5S818 primers 1 [SEQ ID NO:5] and 2 [FL-SEQ ID NO:6], .30 μ M each HUMCSF1PO primers 1 [TMR-SEQ ID NO:33] and 2 [SEQ ID NO:34], .40 μ M each HUMTPOX primers 1 [SEQ ID NO:35] and 2 [TMR-SEQ ID
25 NO:35], .40 μ M each HUMTH01 primers 1 [SEQ ID NO:37] and 2 [TMR-SEQ ID NO:38].

Amplified products were separated by denaturing polyacrylamide gel electrophoresis on a 32 cm gel for 50 min. at 40 W and products were visualized by detection of the fluorescent signals using the
30 FMBIOFluorImager™ (Hitachi Software Engineering, San Bruno, CA).

Reference is made to Figure 18 which displays the amplified fragments of each locus in separate 505 nm and 625 nm scans of the same gel revealing fluorescein-labeled and tetramethyl-rhodamine labeled material, respectively. Lanes 1 to 5 contain DNA samples simultaneously co-amplified for the loci D16S539, D7S820, D13S317, D5S818, HUMCSF1PO ("CSF1PO"), HUMTPOX ("TPOX") and HUMTH01 ("TH01").

EXAMPLE 19

Fluorescent Detection of Multiplex Amplification of Loci D16S539, D7S820, D13S317, D5S818, HUMCSF1PO, HUMTPOX, HUMTH01 and HUMvWFA31

10

In this example, a DNA template was amplified simultaneously at the individual loci D16S539, D7S820, D13S317, D5S818, HUMCSF1PO, HUMTPOX, HUMTH01 and HUMvWFA31 in a single reaction vessel. The PCR amplification was performed in 25 μ l of 1x STR Buffer (50mM KCl, 10mM Tris-HCl (pH 9.0 at 25°C), 0.1% Triton X-100, 1.5mM MgCl₂ and 200 μ M each of dATP, dCTP, dGTP and dTTP) using 5 ng template, and 0.08 U *Taq* DNA Polymerase/ μ l. A Thermal Cycler 480 (Perkin Elmer, Foster City, CA) was employed with the following amplification protocol: 96°C for 2 min., then 10 cycles of 94°C for 1 min., 60°C for 1 min., and 70°C for 1.5 min., followed by 20 cycles of 90°C for 1 min., 60°C for 1 min., 70°C for 1.5 min., followed by 1 cycle of 60°C for 30 min.

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Sixteen amplification primers were used in combination, including .75 μ M each D16S539 primers 1 [SEQ ID NO:29] and 2 [FL-SEQ ID NO:30], .40 μ M each D7S820 primers 1 [SEQ ID NO:1] and 2 [FL-SEQ ID NO:2], .30 μ M each D13S317 primers 1 [SEQ ID NO:3] and 2 [FL-SEQ ID NO:4], .60 μ M each D5S818 primers 1 [SEQ ID NO:5] and 2 [FL-SEQ ID NO:6], .30 μ M each HUMCSF1PO primers 1 [TMR-SEQ ID NO:33] and 2 [SEQ ID NO:34], .40 μ M each HUMTPOX primers 1 [SEQ ID NO:35] and 2 [TMR-SEQ ID NO:36], .40 μ M each HUMTH01 primers 1 [SEQ ID NO:37] and 2 [TMR-

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SEQ ID NO:38], .40 μ M each HUMvWFA31 primers 1 [SEQ ID NO:39] and 2 [TMR-SEQ ID NO:40].

Amplified products were separated by denaturing polyacrylamide gel electrophoresis on a 32 cm gel for 50 min. at 40 W and products were
5 visualized by detection of the fluorescent signals using the FMBIOFluorImager™ (Hitachi Software Engineering, San Bruno, CA).

Reference is made to Figure 19 which displays the amplified fragments of each locus in separate 505 nm and 625 nm scans of the same gel revealing fluorescein-labeled and tetramethyl-rhodamine labeled material,
10 respectively. Lanes 1 to 5 contain DNA samples simultaneously co-amplified for the loci D16S539, D7S820, D13S317, D5S818, HUMCSF1PO ("CSF1PO"), HUMTPOX ("TPOX"), HUMTH01 ("TH01") and HUMvWFA31 ("vWA").

EXAMPLE 20

15 Fluorescent Detection of Multiplex Amplification of
Loci D16S539, D7S820, D13S317, D5S818,
HUMF13A01 and HUMFESFPS

In this example, a DNA template was amplified simultaneously at the individual loci D16S539, D7S820, D13S317, D5S818, HUMF13A01 and
20 HUMFESFPS in a single reaction vessel. The PCR amplification was performed in 25 μ l of 1x STR Buffer (50mM KCl, 10mM Tris-HCl (pH 9.0 at 25°C), 0.1% Triton X-100, 1.5mM MgCl₂ and 200 μ M each of dATP, dCTP, dGTP and dTTP) using 5 ng template, and 0.06 U *Taq* DNA Polymerase/ μ l. A Thermal Cycler 480 (Perkin Elmer, Foster City, CA) was
25 employed with the following amplification protocol: 96°C for 2 min., then 10 cycles of 94°C for 1 min., 60°C for 1 min., and 70°C for 1.5 min., followed by 20 cycles of 90°C for 1 min., 60°C for 1 min., 70°C for 1.5 min., followed by 1 cycle of 60°C for 30 min.

Twelve amplification primers were used in combination, including .75
30 μ M each D16S539 primers 1 [SEQ ID NO:29] and 2 [FL-SEQ ID NO:30], .40

μ M each D7S820 primers 1 [SEQ ID NO:1] and 2 [FL-SEQ ID NO:2], .30 μ M each D13S317 primers 1 [SEQ ID NO:3] and 2 [FL-SEQ ID NO:4], .60 μ M each D5S818 primers 1 [SEQ ID NO:5] and 2 [FL-SEQ ID NO:6], .10 μ M each HUMF13A01 primers 1 [TMR-SEQ ID NO:41] and 2 [SEQ ID NO:42],
5 1.0 μ M each HUMFESFPS primers 1 [TMR-SEQ ID NO:43] and 2 [SEQ ID NO:44].

Amplified products were separated by denaturing polyacrylamide gel electrophoresis on a 32 cm gel for 50 min. at 40 W and products were visualized by detection of the fluorescent signals using the
10 FMBIOFluorImager™ (Hitachi Software Engineering, San Bruno, CA).

Reference is made to Figure 20 which displays the amplified fragments of each locus in separate 505 nm and 625 nm scans of the same gel revealing fluorescein-labeled and tetramethyl-rhodamine labeled material, respectively. Lanes 1 to 5 contain DNA samples simultaneously co-
15 amplified for the loci D16S539, D7S820, D13S317, D5S818, HUMF13A01 ("F13A01") and HUMFESFPS ("FESFPS").

EXAMPLE 21

Fluorescent Detection of Multiplex Amplification of Loci D16S539, D7S820, D13S317, D5S818, HUMF13A01, 20 HUMFESFPS and HUMBFXIII

In this example, a DNA template was amplified simultaneously at the individual loci D16S539, D7S820, D13S317, D5S818, HUMF13A01, HUMFESFPS and HUMBFXIII in a single reaction vessel. The PCR amplification was performed in 25 μ l of 1x STR Buffer (50mM KCl, 10mM
25 Tris-HCl (pH 9.0 at 25°C), 0.1% Triton X-100, 1.5mM MgCl₂ and 200 μ M each of dATP, dCTP, dGTP and dTTP) using 5 ng template, and 0.07 U *Taq* DNA Polymerase/ μ l. A Thermal Cycler 480 (Perkin Elmer, Foster City, CA) was employed with the following amplification protocol: 96°C for 2 min., then 10 cycles of 94°C for 1 min., 60°C for 1 min., and 70°C for 1.5 min.,

followed by 20 cycles of 90°C for 1 min., 60°C for 1 min., 70°C for 1.5 min., followed by 1 cycle of 60°C for 30 min.

Fourteen amplification primers were used in combination, including .75 μ M each D16S539 primers 1 [SEQ ID NO:29] and 2 [FL-SEQ ID NO:30],
5 .40 μ M each D7S820 primers 1 [SEQ ID NO:1] and 2 [FL-SEQ ID NO:2], .30 μ M each D13S317 primers 1 [SEQ ID NO:3] and 2 [FL-SEQ ID NO:4], .60 μ M each D5S818 primers 1 [SEQ ID NO:5] and 2 [FL-SEQ ID NO:6], .10 μ M each HUMF13A01 primers 1 [TMR-SEQ ID NO:41] and 2 [SEQ ID NO:42],
1.0 μ M each HUMFESFPS primers 1 [TMR-SEQ ID NO:43] and 2 [SEQ ID NO:44],
10 .50 μ M each HUMBFXIII primers 1 [TMR-SEQ ID NO:45] and 2 [SEQ ID NO:46].

Amplified products were separated by denaturing polyacrylamide gel electrophoresis on a 32 cm gel for 50 min. at 40 W and products were visualized by detection of the fluorescent signals using the
15 FMBIOFluorImager™ (Hitachi Software Engineering, San Bruno, CA).

Reference is made to Figure 21 which displays the amplified fragments of each locus in separate 505 nm and 625 nm scans of the same gel revealing fluorescein-labeled and tetramethyl-rhodamine labeled material, respectively. Lanes 1 to 5 contain DNA samples simultaneously co-
20 amplified for the loci D16S539, D7S820, D13S317, D5S818, HUMF13A01 ("F13A01"), HUMFESFPS ("FESFPS") and HUMBFXIII ("F13B").

EXAMPLE 22

Fluorescent Detection of Multiplex Amplification of Loci D16S539, D7S820, D13S317, D5S818, HUMF13A01, 25 HUMFESFPS, HUMBFXIII and HUMLIPOI

In this example, a DNA template was amplified simultaneously at the individual loci D16S539, D7S820, D13S317, D5S818, HUMF13A01, HUMFESFPS, HUMBFXIII and HUMLIPOI in a single reaction vessel. The PCR amplification was performed in 25 μ l of 1x STR Buffer (50mM KCl,
30 10mM Tris-HCl (pH 9.0 at 25°C), 0.1% Triton X-100, 1.5mM MgCl₂ and

200 μ M each of dATP, dCTP, dGTP and dTTP) using 5 ng template, and 0.08 U *Taq* DNA Polymerase/ μ l. A Thermal Cycler 480 (Perkin Elmer, Foster City, CA) was employed with the following amplification protocol: 96°C for 2 min., then 10 cycles of 94°C for 1 min., 60°C for 1 min., and 70°C for 1.5 min., followed by 20 cycles of 90°C for 1 min., 60°C for 1 min., 70°C for 1.5 min., followed by 1 cycle of 60°C for 30 min.

Sixteen amplification primers were used in combination, including .75 μ M each D16S539 primers 1 [SEQ ID NO:29] and 2 [FL-SEQ ID NO:30], .40 μ M each D7S820 primers 1 [SEQ ID NO:1] and 2 [FL-SEQ ID NO:2], .30 μ M each D13S317 primers 1 [SEQ ID NO:3] and 2 [FL-SEQ ID NO:4], .60 μ M each D5S818 primers 1 [SEQ ID NO:5] and 2 [FL-SEQ ID NO:6], .10 μ M each HUMF13A01 primers 1 [TMR-SEQ ID NO:41] and 2 [SEQ ID NO:42], 1.0 μ M each HUMFESFPS primers 1 [TMR-SEQ ID NO:43] and 2 [SEQ ID NO:44], .50 μ M each HUMBFXIII primers 1 [TMR-SEQ ID NO:45] and 2 [SEQ ID NO:46], .20 μ M each HUMLIPOL primers 1 [TMR-SEQ ID NO:47] and 2 [SEQ ID NO:48].

Amplified products were separated by denaturing polyacrylamide gel electrophoresis on a 32 cm gel for 50 min. at 40 W and products were visualized by detection of the fluorescent signals using the FMBIOFluorImager™ (Hitachi Software Engineering, San Bruno, CA).

Reference is made to Figure 22 which displays the amplified fragments of each locus in separate 505 nm and 625 nm scans of the same gel revealing fluorescein-labeled and tetramethyl-rhodamine labeled material, respectively. Lanes 1 to 5 contain DNA samples simultaneously co-amplified for the loci D16S539, D7S820, D13S317, D5S818, HUMF13A01 ("HUMF13A01"), HUMFESFPS ("FESFPS"), HUMBFXIII ("F13B") and HUMLIPOL ("LPL").

EXAMPLE 23

Fluorescent Detection of Multiplex Amplification of
Loci D16S539, D7S820, D13S317, D5S818, HUMCSF1PO,
HUMTPOX, HUMTH01 and HUMvWFA31

5 In this example, a DNA template was amplified simultaneously at the individual loci D16S539, D7S820, D13S317, D5S818, HUMCSF1PO, HUMTPOX, HUMTH01 and HUMvWFA31 in a single reaction vessel. The PCR amplification was performed in 25 μ l of 1x STR Buffer (50mM KCl, 10mM Tris-HCl (pH 9.0 at 25°C), 0.1% Triton X-100, 1.5mM MgCl₂ and
10 200 μ M each of dATP, dCTP, dGTP and dTTP) using 5 ng template, and 0.08 U *Taq* DNA Polymerase/ μ l. A Thermal Cycler 480 (Perkin Elmer, Foster City, CA) was employed with the following amplification protocol: 96°C for 2 min., then 10 cycles of 94°C for 1 min., 60°C for 1 min., and 70°C for 1.5 min., followed by 20 cycles of 90°C for 1 min., 60°C for
15 1 min., 70°C for 1.5 min., followed by 1 cycle of 60°C for 30 min.

 Sixteen amplification primers were used in combination, including .75 μ M each D16S539 primers 1 [SEQ ID NO:29] and 2 [TMR-SEQ ID NO:30], .40 μ M each D7S820 primers 1 [SEQ ID NO:1] and 2 [TMR-SEQ ID NO:2], .30 μ M each D13S317 primers 1 [SEQ ID NO:3] and 2 [TMR-SEQ ID NO:4],
20 .60 μ M each D5S818 primers 1 [SEQ ID NO:5] and 2 [TMR-SEQ ID NO:6], .40 μ M each HUMCSF1PO primers 1 [FL-SEQ ID NO:33] and 2 [SEQ ID NO:34], .50 μ M each HUMTPOX primers 1 [SEQ ID NO:35] and 2 [FL-SEQ ID NO:36], .20 μ M each HUMTH01 primers 1 [SEQ ID NO:37] and 2 [FL-SEQ ID NO:38], .55 μ M each HUMvWFA31 primers 1 [SEQ ID NO:39] and
25 2 [FL-SEQ ID NO:40].

 Amplified products were separated by denaturing polyacrylamide gel electrophoresis on a 32 cm gel for 50 min. at 40 W and products were visualized by detection of the fluorescent signals using the FMBIOFluorImager™ (Hitachi Software Engineering, San Bruno, CA).

30 Reference is made to Figure 23 which displays the amplified fragments of each locus in separate 505 nm and 625 nm scans of the same

gel revealing fluorescein-labeled and tetramethyl-rhodamine labeled material, respectively. Lanes 1 to 3 contain DNA samples simultaneously co-amplified for the loci D16S539, D7S820, D13S317, D5S818, HUMCSF1PO ("CSF1PO"), HUMTPOX ("TPOX"), HUMTH01 ("TH01") and HUMvWFA31 ("vWA").

EXAMPLE 24

Fluorescent Detection of Multiplex Amplification of Loci D3S1539, D19S253, D13S317, and D20S481

In this example, a DNA template was amplified simultaneously at the individual loci D3S1539, D19S253, D13S317 and D20S481 in a single reaction vessel. The PCR amplification was performed in 25 μ l of 1x STR Buffer (50mM KCl, 10mM Tris-HCl (pH 9.0 at 25°C), 0.1% Triton X-100, 1.5mM MgCl₂ and 200 μ M each of dATP, dCTP, dGTP and dTTP) using 5 ng template, and 0.04 U *Taq* DNA Polymerase/ μ l. A Thermal Cycler 480 (Perkin Elmer, Foster City, CA) was employed with the following amplification protocol: 96°C for 2 min., then 10 cycles of 94°C for 1 min., 60°C for 1 min., and 70°C for 1.5 min., followed by 20 cycles of 90°C for 1 min., 60°C for 1 min., 70°C for 1.5 min., followed by 1 cycle of 60°C for 30 min.

Eight amplification primers were used in combination, including .75 μ M each D3S1539 primers 1 [SEQ ID NO:7] and 2 [FL-SEQ ID NO:49], .75 μ M each D19S253 primers 1 [FL-SEQ ID NO:50] and 2 [SEQ ID NO:51], .50 μ M each D13S317 primers 1 [SEQ ID NO:3] and 2 [FL-SEQ ID NO:4], .50 μ M each D20S481 primers 1 [SEQ ID NO:52] and 2 [FL-SEQ ID NO:53].

Amplified products were separated by denaturing polyacrylamide gel electrophoresis on a 32 cm gel for 50 min. at 40 W and products were visualized by detection of the fluorescent signals using the FluorImager™ fluorescent scanner (Molecular Dynamics, Sunnyvale, CA).

Reference is made to Figure 24 which displays the amplified fragments of each locus. Lanes 1 to 5 contain DNA samples simultaneously co-amplified for the loci D3S1539, D19S253, D13S317 and D20S481.

EXAMPLE 25

5 Fluorescent Detection of Multiplex Amplification of
 Loci D10S1239, D9S930, D4S2368, and D20S481

In this example, a DNA template was amplified simultaneously at the individual loci D10S1239, D9S930, D4S2368 and D20S481 in a single reaction vessel. The PCR amplification was performed in 25 μ l of 1x STR
10 Buffer (50mM KCl, 10mM Tris-HCl (pH 9.0 at 25°C), 0.1% Triton X-100, 1.5mM MgCl₂ and 200 μ M each of dATP, dCTP, dGTP and dTTP) using 5 ng template, and 0.04 U *Taq* DNA Polymerase/ μ l. A Thermal Cycler 480 (Perkin Elmer, Foster City, CA) was employed with the following amplification protocol: 96°C for 2 min., then 10 cycles of 94°C for 1 min.,
15 60°C for 1 min., and 70°C for 1.5 min., followed by 20 cycles of 90°C for 1 min., 60°C for 1 min., 70°C for 1.5 min., followed by 1 cycle of 60°C for 30 min.

Eight amplification primers were used in combination, including .30 μ M each D10S1239 primers 1 [FL-SEQ ID NO:15] and 2 [SEQ ID NO:54],
20 .40 μ M each D9S930 primers 1 [SEQ ID NO:55] and 2 [FL-SEQ ID NO:14], .50 μ M each D4S2368 primers 1 [SEQ ID NO:56] and 2 [FL-SEQ ID NO:57], .50 μ M each D20S481 primers 1 [SEQ ID NO:52] and 2 [FL-SEQ ID NO:53].

Amplified products were separated by denaturing polyacrylamide gel electrophoresis on a 32 cm gel for 50 min. at 40 W and products were
25 visualized by detection of the fluorescent signals using the FluorImager™ fluorescent scanner (Molecular Dynamics, Sunnyvale, CA).

Reference is made to Figure 25 which displays the amplified fragments of each locus. Lanes 1 to 5 contain DNA samples simultaneously co-amplified for the loci D10S1239, D9S930, D4S2368 and D20S481.

EXAMPLE 26

Silver Detection of Multiplex Amplification of
Loci D16S539, D7S820 and D13S317

In this example, a DNA template was amplified simultaneously at the
5 individual loci, D16S539, D7S820, and D13S317 in a single reaction
vessel. The PCR amplification was performed in 25 μ l of 1x STR Buffer
(50mM KCl, 10mM Tris-HCl (pH 9.0 at 25°C), 0.1% Triton X-100, 1.5mM
MgCl₂ and 200 μ M each of dATP, dCTP, dGTP and dTTP) using 5 to 25 ng
template, and 0.03 U *Taq* DNA Polymerase/ μ l. A Thermal Cycler 480
10 (Perkin Elmer, Foster City, CA) was employed with the following
amplification protocol: 96°C for 2 min., then 10 cycles of 94°C for 1 min.,
60°C for 1 min., and 70°C for 1.5 min., followed by 20 cycles of 90°C for
1 min., 60°C for 1 min., 70°C for 1.5 min, followed by 1 cycle of 60°C for
30 min.

15 Six amplification primers were used in combination, including 0.5 μ M
each D16S539 primers 1 [SEQ ID NO:29] and 2 [SEQ ID NO:58], 0.5 μ M
each D7S820 primers 1 [SEQ ID NO:1] and 2 [SEQ ID NO:2], 0.5 μ M each
D13S317 primers 1 [SEQ ID NO:3] and 2 [SEQ ID NO:4].

20 Amplified products were separated by denaturing polyacrylamide gel
electrophoresis on a 32 cm gel for 50 min. at 40 W and products were
visualized by silver stain analysis according to the protocol of Bassam et
al.(1991).

Reference is made to Figure 26 which displays the amplified
fragments of each locus. Lanes 1 to 4 contain DNA samples simultaneously
25 co-amplified for the loci D16S539, D7S820, and D13S317 and lane 5
displays a sample without DNA template subjected to the same procedures,
i.e., a negative control.

EXAMPLE 27

Silver Detection of Multiplex Amplification of
Loci D16S539, D7S820, D13S317 and HUMvWFA31

In this example, a DNA template was amplified simultaneously at the individual loci, D16S539, D7S820, D13S317 and HUMvWFA31 in a single reaction vessel. The PCR amplification was performed in 25 μ l of 1x STR Buffer (50mM KCl, 10mM Tris-HCl (pH 9.0 at 25°C), 0.1% Triton X-100, 1.5mM MgCl₂ and 200 μ M each of dATP, dCTP, dGTP and dTTP) using 5 ng template, and 0.04 U *Taq* DNA Polymerase/ μ l. A Thermal Cycler 480 (Perkin Elmer, Foster City, CA) was employed with the following amplification protocol: 96°C for 2 min., then 10 cycles of 94°C for 1 min., 60°C for 1 min., and 70°C for 1.5 min., followed by 20 cycles of 90°C for 1 min., 60°C for 1 min., 70°C for 1.5 min, followed by 1 cycle of 60°C for 30 min.

Eight amplification primers were used in combination, including 0.3 μ M each D16S539 primers 1 [SEQ ID NO:29] and 2 [SEQ ID NO:30], 0.3 μ M each D7S820 primers 1 [SEQ ID NO:1] and 2 [SEQ ID NO:2], 0.5 μ M each D13S317 primers 1 [SEQ ID NO:3] and 2 [SEQ ID NO:4], 0.5 μ M each HUMvWFA31 primers 1 [SEQ ID NO:59] and 2 [SEQ ID NO:60].

Amplified products were separated by denaturing polyacrylamide gel electrophoresis on a 32 cm gel for 50 min. at 40 W and products were visualized by silver stain analysis according to the protocol of Bassam et al.(1991).

Reference is made to Figure 27 which displays the amplified fragments of each locus. Lanes 1 to 3 contain DNA samples simultaneously co-amplified for the loci D16S539, D7S820, D13S317 and HUMvWFA31 ("vWA").

EXAMPLE 28

Silver Detection of Multiplex Amplification of
Loci D10S1239, D9S930, and D13S317

In this example, a DNA template was amplified simultaneously at the individual loci, D10S1239, D9S930, and D13S317 in a single reaction vessel. The PCR amplification was performed in 25 μ l of 1x STR Buffer (50mM KCl, 10mM Tris-HCl (pH 9.0 at 25°C), 0.1% Triton X-100, 1.5mM MgCl₂ and 200 μ M each of dATP, dCTP, dGTP and dTTP) using undetermined ng template, and 0.03 U *Taq* DNA Polymerase/ μ l. A Thermal
Cycler 480 (Perkin Elmer, Foster City, CA) was employed with the following amplification protocol: 96°C for 2 min., then 10 cycles of 94°C for 1 min., 60°C for 1 min., and 70°C for 1.5 min., followed by 20 cycles of 90°C for 1 min., 60°C for 1 min., 70°C for 1.5 minutes.

Six amplification primers were used in combination, including 1.0 μ M each D10S1239 primers 1 [SEQ ID NO:15] and 2 [SEQ ID NO:54], 0.3 μ M each D9S930 primers 1 [SEQ ID NO:55] and 2 [SEQ ID NO:61], 0.5 μ M each D13S317 primers 1 [SEQ ID NO:3] and 2 [SEQ ID NO:4].

Amplified products were separated by denaturing polyacrylamide gel electrophoresis on a 40 cm gel for 60 min. at 60 W and products were visualized by silver stain analysis according to the protocol of Bassam et al.(1991).

Reference is made to Figure 28 which displays the amplified fragments of each locus. Lanes 1 to 3 contain DNA samples simultaneously co-amplified for the loci D10S1239, D9S930, and D13S317.

EXAMPLE 29

Silver Detection of Multiplex Amplification of
Loci D10S1239, D9S930, and D4S2368

In this example, a DNA template was amplified simultaneously at the individual loci, D10S1239, D9S930, and D4S2368 in a single reaction

vessel. The PCR amplification was performed in 25 μ l of 1x STR Buffer (50mM KCl, 10mM Tris-HCl (pH 9.0 at 25°C), 0.1% Triton X-100, 1.5mM MgCl₂ and 200 μ M each of dATP, dCTP, dGTP and dTTP) using undetermined ng template, and 0.03 U *Taq* DNA Polymerase/ μ l. A Thermal
5 Cycler 480 (Perkin Elmer, Foster City, CA) was employed with the following amplification protocol: 96°C for 2 min., then 10 cycles of 94°C for 1 min., 60°C for 1 min., and 70°C for 1.5 min., followed by 20 cycles of 90°C for 1 min., 60°C for 1 min., 70°C for 1.5 min., followed by 1 cycle of 60°C for 30 min.

10 Six amplification primers were used in combination, including 1.0 μ M each D10S1239 primers 1 [SEQ ID NO:15] and 2 [SEQ ID NO:54], 0.3 μ M each D9S930 primers 1 [SEQ ID NO:55] and 2 [SEQ ID NO:61], 0.15 μ M each D4S2368 primers 1 [SEQ ID NO:56] and 2 [SEQ ID NO:57].

15 Amplified products were separated by denaturing polyacrylamide gel electrophoresis on a 40 cm gel for 60 min. at 60 W and products were visualized by silver stain analysis according to the protocol of Bassam et al. (1991).

Reference is made to Figure 29 which displays the amplified fragments of each locus. Lanes 1 to 6 contain DNA samples simultaneously
20 co-amplified for the loci D10S1239, D9S930, and D4S2368.

EXAMPLE 30

Silver Detection of Multiplex Amplification of Loci D10S1239, D9S930, D4S2368 and D20S481

In this example, a DNA template was amplified simultaneously at the
25 individual loci, D10S1239, D9S930, D4S2368 and D20S481 in a single reaction vessel. The PCR amplification was performed in 25 μ l of 1x STR Buffer (50mM KCl, 10mM Tris-HCl (pH 9.0 at 25°C), 0.1% Triton X-100, 1.5mM MgCl₂ and 200 μ M each of dATP, dCTP, dGTP and dTTP) using undetermined ng template, and 0.04 U *Taq* DNA Polymerase/ μ l. A Thermal
30 Cycler 480 (Perkin Elmer, Foster City, CA) was employed with the following

amplification protocol: 96°C for 2 min., then 10 cycles of 94°C for 1 min., 60°C for 1 min., and 70°C for 1.5 min., followed by 20 cycles of 90°C for 1 min., 60°C for 1 min., 70°C for 1.5 min., followed by 1 cycle of 60°C for 30 min.

5 Eight amplification primers were used in combination, including 1.0 μ M each D10S1239 primers 1 [SEQ ID NO:15] and 2 [SEQ ID NO:54], 4.0 μ M each D9S930 primers 1 [SEQ ID NO:55] and 2 [SEQ ID NO:14], 0.2 μ M each D4S2368 primers 1 [SEQ ID NO:56] and 2 [SEQ ID NO:57] and 0.2 μ M each D20S481 primers 1 [SEQ ID NO:52] and 2 [SEQ ID NO:53].

10 Amplified products were separated by denaturing polyacrylamide gel electrophoresis on a 40 cm gel for 67 min. at 60 W and products were visualized by silver stain analysis according to the protocol of Bassam et al.(1991).

15 Reference is made to Figure 30 which displays the amplified fragments of each locus. Lanes 1 to 4 contain DNA samples simultaneously co-amplified for the loci D10S1239, D9S930, D4S2368 and D20S481.

EXAMPLE 31

Silver Detection of Multiplex Amplification of Loci D3S1539, D19S253 and D13S317

20 In this example, a DNA template was amplified simultaneously at the individual loci, D3S1539, D19S253 and D13S317 in a single reaction vessel. The PCR amplification was performed in 25 μ l of 1x STR Buffer (50mM KCl, 10mM Tris-HCl (pH 9.0 at 25°C), 0.1% Triton X-100, 1.5mM MgCl₂ and 200 μ M each of dATP, dCTP, dGTP and dTTP) using 5.0 ng
25 template, and 0.03 U *Taq* DNA Polymerase/ μ l. A Thermal Cycler 480 (Perkin Elmer, Foster City, CA) was employed with the following amplification protocol: 96°C for 2 min., then 10 cycles of 94°C for 1 min., 60°C for 1 min., and 70°C for 1.5 min., followed by 20 cycles of 90°C for 1 min., 60°C for 1 min., 70°C for 1.5 min.

Six amplification primers were used in combination, including 1.0 μ M each D3S1539 primers 1 [SEQ ID NO:7] and 2 [SEQ ID NO:49], 1.0 μ M each D19S253 primers 1 [SEQ ID NO:50] and 2 [SEQ ID NO:51], 0.5 μ M each D13S317 primers 1 [SEQ ID NO:3] and 2 [SEQ ID NO:4].

5 Amplified products were separated by denaturing polyacrylamide gel electrophoresis on a 40 cm gel for 65 min. at 60 W and products were visualized by silver stain analysis according to the protocol of Bassam et al.(1991).

Reference is made to Figure 31 which displays the amplified
10 fragments of each locus. Lanes 1 to 4 contain DNA samples simultaneously co-amplified for the loci D3S1539, D19S253 and D13S317 and lane 5 displays a sample without DNA template subjected to the same procedures, i.e., a negative control.

EXAMPLE 32

15 Silver Detection of Multiplex Amplification of
Loci D3S1539, D19S253, D4S2368 and D20S481

In this example, a DNA template was amplified simultaneously at the individual loci, D3S1539, D19S253, D4S2368 and D20S481 in a single reaction vessel. The PCR amplification was performed in 25 μ l of 1x STR
20 Buffer (50mM KCl, 10mM Tris-HCl (pH 9.0 at 25°C), 0.1% Triton X-100, 1.5mM MgCl₂ and 200 μ M each of dATP, dCTP, dGTP and dTTP) using 5ng template, and 0.04 U *Taq* DNA Polymerase/ μ l. A Thermal Cycler 480 (Perkin Elmer, Foster City, CA) was employed with the following amplification protocol: 96°C for 2 min., then 10 cycles of 94°C for 1 min.,
25 60°C for 1 min., and 70°C for 1.5 min., followed by 20 cycles of 90°C for 1 min., 60°C for 1 min., 70°C for 1.5 min.

Eight amplification primers were used in combination, including 1.0 μ M each D3S1539 primers 1 [SEQ ID NO:7] and 2 [SEQ ID NO:49], 0.5 μ M each D19S253 primers 1 [SEQ ID NO:50] and 2 [SEQ ID NO:51], 0.1 μ M

each D4S2368 primers 1 [SEQ ID NO:56] and 2 [SEQ ID NO:57]], 0.1 μ M each D20S481 primers 1 [SEQ ID NO:52] and 2 [SEQ ID NO:53].

Amplified products were separated by denaturing polyacrylamide gel electrophoresis on a 40 cm gel for 65 min. at 60 W and products were visualized by silver stain analysis according to the protocol of Bassam et al. (1991).

Reference is made to Figure 32 which displays the amplified fragments of each locus. Lanes 1 to 4 contain DNA samples simultaneously co-amplified for the loci D3S1539, D19S253, D4S2368 and D20S481 and lane 5 displays a sample without DNA template subjected to the same procedures, i.e., a negative control.

EXAMPLE 33

Silver Detection of Multiplex Amplification of Loci D3S1539, D19S253, D13S317 and D20S481

In this example, a DNA template was amplified simultaneously at the individual loci, D3S1539, D19S253, D13S317 and D20S481 in a single reaction vessel. The PCR amplification was performed in 25 μ l of 1x STR Buffer (50mM KCl, 10mM Tris-HCl (pH 9.0 at 25°C), 0.1% Triton X-100, 1.5mM MgCl₂ and 200 μ M each of dATP, dCTP, dGTP and dTTP) using 0.5 to 250 ng template, and 0.04 U *Taq* DNA Polymerase/ μ l. A Thermal Cycler 480 (Perkin Elmer, Foster City, CA) was employed with the following amplification protocol: 96°C for 2 min., then 10 cycles of 94°C for 1 min., 60°C for 1 min., and 70°C for 1.5 min., followed by 20 cycles of 90°C for 1 min., 60°C for 1 min., 70°C for 1.5 min., followed by 1 cycle of 60°C for 30 min.

Eight amplification primers were used in combination, including 0.5 μ M each D3S1539 primers 1 [SEQ ID NO:7] and 2 [SEQ ID NO:49], 0.5 μ M each D19S253 primers 1 [SEQ ID NO:50] and 2 [SEQ ID NO:51], 0.5 μ M each D13S317 primers 1 [SEQ ID NO:3] and 2 [SEQ ID NO:4] , 0.2 μ M each D20S481 primers 1 [SEQ ID NO:52] and 2 [SEQ ID NO:53].

Amplified products were separated by denaturing polyacrylamide gel electrophoresis on a 40 cm gel for 68 min. at 60 W and products were visualized by silver stain analysis according to the protocol of Bassam et al.(1991).

Reference is made to Figure 33 which displays the amplified fragments of each locus. Lanes 1 to 5 contain DNA samples simultaneously co-amplified for the loci D3S1539, D19S253, D13S317 and D20S481 and lane 6 displays a sample without DNA template subjected to the same procedures, i.e., a negative control.

10

EXAMPLE 34

Silver Detection of Multiplex Amplification of Loci D10S1239, D9S930 and D20S481

In this example, a DNA template was amplified simultaneously at the individual loci, D10S1239, D9S930 and D20S481 in a single reaction vessel. The PCR amplification was performed in 25 μ l of 1x STR Buffer (50mM KCl, 10mM Tris-HCl (pH 9.0 at 25°C), 0.1% Triton X-100, 1.5mM MgCl₂ and 200 μ M each of dATP, dCTP, dGTP and dTTP) using 0.5 to 250 ng template, and 0.03 U *Taq* DNA Polymerase/ μ l. A Thermal Cycler 480 (Perkin Elmer, Foster City, CA) was employed with the following amplification protocol: 96°C for 2 min., then 10 cycles of 94°C for 1 min., 60°C for 1 min., and 70°C for 1.5 min., followed by 20 cycles of 90°C for 1 min., 60°C for 1 min., 70°C for 1.5 min., followed by 1 cycle of 60°C for 30 min.

25 Six amplification primers were used in combination, including 1.0 μ M each D10S1239 primers 1 [SEQ ID NO:15] and 2 [SEQ ID NO:54], 4.0 μ M each D9S930 primers 1 [SEQ ID NO:55] and 2 [SEQ ID NO:14], and 0.2 μ M each D20S481 primers 1 [SEQ ID NO:52] and 2 [SEQ ID NO:53].

Amplified products were separated by denaturing polyacrylamide gel electrophoresis on a 40 cm gel for 68 min. at 60 W and products were

visualized by silver stain analysis according to the protocol of Bassam et al.(1991).

Reference is made to Figure 34 which displays the amplified fragments of each locus. Lanes 1 to 5 contain DNA samples simultaneously co-amplified for the loci D10S1239, D9S930 and D20S481 and lane 6 displays a sample without DNA template subjected to the same procedures, i.e., a negative control.

EXAMPLE 35

Silver Detection of Multiplex Amplification of Loci D10S1239, D4S2368 and D20S481

In this example, a DNA template was amplified simultaneously at the individual loci, D10S1239, D4S2368 and D20S481 in a single reaction vessel. The PCR amplification was performed in 25 μ l of 1x STR Buffer (50mM KCl, 10mM Tris-HCl (pH 9.0 at 25°C), 0.1% Triton X-100, 1.5mM MgCl₂ and 200 μ M each of dATP, dCTP, dGTP and dTTP) using 0.5 to 250 ng template, and 0.03 U *Taq* DNA Polymerase/ μ l. A Thermal Cycler 480 (Perkin Elmer, Foster City, CA) was employed with the following amplification protocol: 96°C for 2 min., then 10 cycles of 94°C for 1 min., 60°C for 1 min., and 70°C for 1.5 min., followed by 20 cycles of 90°C for 1 min., 60°C for 1 min., 70°C for 1.5 min., followed by 1 cycle of 60°C for 30 min.

Six amplification primers were used in combination, including 1.0 μ M each D10S1239 primers 1 [SEQ ID NO:15] and 2 [SEQ ID NO:54], 0.2 μ M each D4S2368 primers 1 [SEQ ID NO:56] and 2 [SEQ ID NO:57], and 0.2 μ M each D20S481 primers 1 [SEQ ID NO:52] and 2 [SEQ ID NO:53].

Amplified products were separated by denaturing polyacrylamide gel electrophoresis on a 40 cm gel for 68 min. at 60 W and products were visualized by silver stain analysis according to the protocol of Bassam et al.(1991):

Reference is made to Figure 35 which displays the amplified fragments of each locus. Lanes 1 to 5 contain DNA samples simultaneously co-amplified for the loci D10S1239, D4S2368 and D20S481 and lane 6 displays a sample without DNA template subjected to the same procedures,
5 i.e., a negative control.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Schumm, James W.
- (ii) TITLE OF INVENTION: Multiplex Amplification of
Short Tandem Repeat Loci
- (iii) NUMBER OF SEQUENCES: 61
- (iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Promega Corporation
(B) STREET: 2800 Woods Hollow Road
(C) CITY: Madison
(D) STATE: Wisconsin
(E) COUNTRY: U.S.A.
(F) ZIP: 53711-5399
- (v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Diskette - 3.5 inch, 1.44 Mb
(B) COMPUTER: IBM compatible PC
(C) OPERATING SYSTEM: DOS, version 6.0
(D) SOFTWARE: WordPerfect 5.1 (DOS text
format)
- (vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: 08/632,575
(B) FILING DATE: 04/15/96
(C) CLASSIFICATION: 435
- (vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 08/316,544
(B) FILING DATE: 09/30/94
- (2) INFORMATION FOR SEQ ID NO:1
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
- (ii) MOLECULE TYPE: Human Genomic DNA
- (iii) HYPOTHETICAL: no
- (viii) POSITION IN GENOME:
(B) MAP POSITION: D7S820
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GAACACTTGT CATAGTTTAG AACG

24

(2) INFORMATION FOR SEQ ID NO:2

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single

-70-

(D) TOPOLOGY: Linear

(viii) POSITION IN GENOME:
(B) MAP POSITION: D7S820

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

5 CTGAGGTATC AAAAAGTCAG AGG 23

(2) INFORMATION FOR SEQ ID NO:3

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Nucleic Acid

10 (C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(viii) POSITION IN GENOME:
(B) MAP POSITION: D13S317

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

15 ACAGAAGTCT GGGATGTGGA 20

(2) INFORMATION FOR SEQ ID NO:4

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Nucleic Acid

20 (C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(viii) POSITION IN GENOME:
(B) MAP POSITION: D13S317

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

25 GCCCAAAAAG ACAGACAGAA 20

(2) INFORMATION FOR SEQ ID NO:5

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Nucleic Acid

30 (C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(viii) POSITION IN GENOME:
(B) MAP POSITION: D5S818

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

35 GGGTGATTTT CCTCTTTGGT 20

(2) INFORMATION FOR SEQ ID NO:6

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Nucleic Acid

40 (C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

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- (viii) POSITION IN GENOME:
(B) MAP POSITION: D5S818
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:
TGATTCCAAT CATAGCCACA 20
- 5 (2) INFORMATION FOR SEQ ID NO:7
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
10 (D) TOPOLOGY: Linear
- (viii) POSITION IN GENOME:
(B) MAP POSITION: D3S1539
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:
TCTCTTTCCA TTACTCTCTC CATAGC 26
- 15 (2) INFORMATION FOR SEQ ID NO:8
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
20 (D) TOPOLOGY: Linear
- (viii) POSITION IN GENOME:
(B) MAP POSITION: D3S1539
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:
AGTGCTGTTT TAGCTTCCAG GA 22
- 25 (2) INFORMATION FOR SEQ ID NO:9
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
30 (D) TOPOLOGY: Linear
- (viii) POSITION IN GENOME:
(B) MAP POSITION: D17S1298
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:
GTAGGTCTTT TGGTTGCCAG TATG 24
- 35 (2) INFORMATION FOR SEQ ID NO:10
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
40 (D) TOPOLOGY: Linear
- (viii) POSITION IN GENOME:

-72-

(B) MAP POSITION: D17S1298

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

TGTCAGTAAA CCTGTGACCT GAGT

24

(2) INFORMATION FOR SEQ ID NO:11

5 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

10 (viii) POSITION IN GENOME:

(B) MAP POSITION: D20S481

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

AATGGTGAGA AATGGGTTAT GAGTGC

26

(2) INFORMATION FOR SEQ ID NO:12

15 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

20 (viii) POSITION IN GENOME:

(B) MAP POSITION: D20S481

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

TTTCCGGCTT TGTGTCATAA AACAG

25

(2) INFORMATION FOR SEQ ID NO:13

25 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

30 (viii) POSITION IN GENOME:

(B) MAP POSITION: D9S930

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

TGGACAACAG AGTGAGATGC

20

(2) INFORMATION FOR SEQ ID NO:14

35 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

40 (viii) POSITION IN GENOME:

(B) MAP POSITION: D9S930

-73-

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:
GCTATGGGAA TTACAAGCAG GAA 23
- (2) INFORMATION FOR SEQ ID NO:15
(i) SEQUENCE CHARACTERISTICS:
5 (A) LENGTH: 26
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(viii) POSITION IN GENOME:
10 (B) MAP POSITION: D10S1239
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:
CTTTGAAATG GACCCCTAGC TAATGT 26
- (2) INFORMATION FOR SEQ ID NO:16
(i) SEQUENCE CHARACTERISTICS:
15 (A) LENGTH: 21
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(viii) POSITION IN GENOME:
20 (B) MAP POSITION: D10S1239
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:
CACCCCTGTCC CCAGCTATCT G 21
- (2) INFORMATION FOR SEQ ID NO:17
(i) SEQUENCE CHARACTERISTICS:
25 (A) LENGTH: 19
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(viii) POSITION IN GENOME:
30 (B) MAP POSITION: D14S118
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:
CAGCTTGGGC AACATAGGG 19
- (2) INFORMATION FOR SEQ ID NO:18
(i) SEQUENCE CHARACTERISTICS:
35 (A) LENGTH: 24
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(viii) POSITION IN GENOME:
40 (B) MAP POSITION: D14S118
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

-74-

- CAAACCTCCTG AGGTCAAACA ATCC 24
- (2) INFORMATION FOR SEQ ID NO:19
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 21
- (B) TYPE: Nucleic Acid
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear
- (viii) POSITION IN GENOME:
- (B) MAP POSITION: D14S562
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:
- CTTGGAGGGT GGGGTGGCTA A 21
- (2) INFORMATION FOR SEQ ID NO:20
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 24
- (B) TYPE: Nucleic Acid
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear
- (viii) POSITION IN GENOME:
- (B) MAP POSITION: D14S562
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:
- CGAAATTTTG TTGCCTTGCT CTGG 24
- (2) INFORMATION FOR SEQ ID NO:21
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 21
- (B) TYPE: Nucleic Acid
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear
- (viii) POSITION IN GENOME:
- (B) MAP POSITION: D14S548
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:
- CCTGGGCAAC AGAGTGAGAC T 21
- (2) INFORMATION FOR SEQ ID NO:22
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 24
- (B) TYPE: Nucleic Acid
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear
- (viii) POSITION IN GENOME:
- (B) MAP POSITION: D14S548
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:
- ACCCAGCTTT AACAGTTTGT GCTT 24

- (2) INFORMATION FOR SEQ ID NO:23
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(viii) POSITION IN GENOME:
(B) MAP POSITION: D16S490
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:
GGCGGACAC AGAATGTAAA ATC- 23
- (2) INFORMATION FOR SEQ ID NO:24
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(viii) POSITION IN GENOME:
(B) MAP POSITION: D16S490
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:
AAACCCAAAT AGATGACAGG CACA 24
- (2) INFORMATION FOR SEQ ID NO:25
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(viii) POSITION IN GENOME:
(B) MAP POSITION: D16S753
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:
GCACTCCAGG CTGAATGACA GAAC 24
- (2) INFORMATION FOR SEQ ID NO:26
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(viii) POSITION IN GENOME:
(B) MAP POSITION: D16S753
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:
GCAGTGCCGC CTATTTTGT GAAT 24
- (2) INFORMATION FOR SEQ ID NO:27

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- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
- (viii) POSITION IN GENOME:
(B) MAP POSITION: D17S1299
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:
ACCCTGATGA GATAGCACTT GAGC 24
- (2) INFORMATION FOR SEQ ID NO:28
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
- (viii) POSITION IN GENOME:
(B) MAP POSITION: D17S1299
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:
CACTGTGTGG AGGTGTAGCA GAGA 24
- (2) INFORMATION FOR SEQ ID NO:29
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
- (viii) POSITION IN GENOME:
(B) MAP POSITION: D16S539
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:
GGGGGTCTAA GAGCTTGTA AAAG 24
- (2) INFORMATION FOR SEQ ID NO:30
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
- (viii) POSITION IN GENOME:
(B) MAP POSITION: D16S539
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:
TGTGCATCTG TAAGCATGTA TCTATC 26
- (2) INFORMATION FOR SEQ ID NO:31
(i) SEQUENCE CHARACTERISTICS:

-77-

- (A) LENGTH: 23
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
- 5 (viii) POSITION IN GENOME:
(B) MAP POSITION: D22S683
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:
CGAAGGTTGC ATTGAGCCAA GAT 23
- (2) INFORMATION FOR SEQ ID NO:32
10 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
- 15 (viii) POSITION IN GENOME:
(B) MAP POSITION: D22S683
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:
TGGTGGAAAT GCCTCATGTA GAAA 24
- (2) INFORMATION FOR SEQ ID NO:33
20 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
- 25 (viii) POSITION IN GENOME:
(B) MAP POSITION: HUMCSF1PO
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:
AACCTGAGTC TGCCAAGGAC TAGC 24
- (2) INFORMATION FOR SEQ ID NO:34
30 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
- 35 (viii) POSITION IN GENOME:
(B) MAP POSITION: HUMCSF1PO
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:
TTCCACACAC CACTGGCCAT CTTC 24
- (2) INFORMATION FOR SEQ ID NO:35
40 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24

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- (B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
- (viii) POSITION IN GENOME:
5 (B) MAP POSITION: HUMTPOX
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:
ACTGGCACAG AACAGGCACT TAGG 24
- (2) INFORMATION FOR SEQ ID NO:36
10 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
- (viii) POSITION IN GENOME:
15 (B) MAP POSITION: HUMTPOX
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:
GGAGGAACTG GGAACCACAC AGGT 24
- (2) INFORMATION FOR SEQ ID NO:37
20 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
- (viii) POSITION IN GENOME:
25 (B) MAP POSITION: HUMTHO1
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:
ATTCAAAGGG TATCTGGGCT CTGG 24
- (2) INFORMATION FOR SEQ ID NO:38
30 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
- (viii) POSITION IN GENOME:
35 (B) MAP POSITION: HUMTHO1
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:
GTGGGCTGAA AAGCTCCCGA TTAT 24
- (2) INFORMATION FOR SEQ ID NO:39
40 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29

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- (B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
- (viii) POSITION IN GENOME:
5 (B) MAP POSITION: HUMvWFA31
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:
GAAAGCCCTA GTGGATGATA AGAATAATC 29
- (2) INFORMATION FOR SEQ ID NO:40
10 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
- (viii) POSITION IN GENOME:
15 (B) MAP POSITION: HUMvWFA31
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:
GGACAGATGA TAAATACATA GGATGGATGG 30
- (2) INFORMATION FOR SEQ ID NO:41
20 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
- (viii) POSITION IN GENOME:
25 (B) MAP POSITION: HUMF13A01
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:
GAGGTTGCAC TCCAGCCTTT GCAA 24
- (2) INFORMATION FOR SEQ ID NO:42
30 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
- (viii) POSITION IN GENOME:
35 (B) MAP POSITION: HUMF13A01
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:
TTCCTGAATC ATCCCAGAGC CACA 24
- (2) INFORMATION FOR SEQ ID NO:43
40 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23

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- (B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
- (viii) POSITION IN GENOME:
5 (B) MAP POSITION: HUMFESFPS
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:
GCTGTTAATT CATGTAGGGA AGG 23
- (2) INFORMATION FOR SEQ ID NO:44
(i) SEQUENCE CHARACTERISTICS:
10 (A) LENGTH: 24
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
- (viii) POSITION IN GENOME:
15 (B) MAP POSITION: HUMFESFPS
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:
GTAGTCCCAG CTA CTGCT ACTC 24
- (2) INFORMATION FOR SEQ ID NO:45
(i) SEQUENCE CHARACTERISTICS:
20 (A) LENGTH: 20
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
- (viii) POSITION IN GENOME:
25 (B) MAP POSITION: HUMBFXIII
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:
TGAGGTGGTG TACTACCATA 20
- (2) INFORMATION FOR SEQ ID NO:46
(i) SEQUENCE CHARACTERISTICS:
30 (A) LENGTH: 20
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
- (viii) POSITION IN GENOME:
35 (B) MAP POSITION: HUMBFXIII
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:
GATCATGCCA TTGCACTCTA 20
- (2) INFORMATION FOR SEQ ID NO:47
(i) SEQUENCE CHARACTERISTICS:
40 (A) LENGTH: 24

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- (B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
- (viii) POSITION IN GENOME:
5 (B) MAP POSITION: HUMLIPOL
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:
CTGACCAAGG ATAGTGGGAT ATAG 24
- (2) INFORMATION FOR SEQ ID NO:48
(i) SEQUENCE CHARACTERISTICS:
10 (A) LENGTH: 23
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
- (viii) POSITION IN GENOME:
15 (B) MAP POSITION: HUMLIPOL
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:
GGTAACTGAG CGAGACTGTG TCT 23
- (2) INFORMATION FOR SEQ ID NO:49
(i) SEQUENCE CHARACTERISTICS:
20 (A) LENGTH: 18
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
- (viii) POSITION IN GENOME:
25 (B) MAP POSITION: D3S1539
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:
CCACCCTTTC AGCACCAG 18
- (2) INFORMATION FOR SEQ ID NO:50
(i) SEQUENCE CHARACTERISTICS:
30 (A) LENGTH: 20
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
- (viii) POSITION IN GENOME:
35 (B) MAP POSITION: D19S253
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:
ATAGACAGAC AGACGGACTG 20
- (2) INFORMATION FOR SEQ ID NO:51
(i) SEQUENCE CHARACTERISTICS:
40 (A) LENGTH: 19

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(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

5 (viii) POSITION IN GENOME:
(B) MAP POSITION: D19S253

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

GGGAGTGGAG ATTACCCCT

19

(2) INFORMATION FOR SEQ ID NO:52

10 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

15 (viii) POSITION IN GENOME:
(B) MAP POSITION: D20S481

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

AAAGCTCTCT GAAGCAGGTG T

21

(2) INFORMATION FOR SEQ ID NO:53

20 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

25 (viii) POSITION IN GENOME:
(B) MAP POSITION: D20S481

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

CAGATTGCAC TAGAAAGAGA GGAA

24

(2) INFORMATION FOR SEQ ID NO:54

30 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

35 (viii) POSITION IN GENOME:
(B) MAP POSITION: D10S1239

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

CACCCTGTCC CCAGCTATCT GGA

23

(2) INFORMATION FOR SEQ ID NO:55

40 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27

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- (B) TYPE: Nucleic Acid
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

- 5 (viii) POSITION IN GENOME:
(B) MAP POSITION: D9S930

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

AGTTGAATCT TGAGTCTCTC AGAGTCA

27

- (2) INFORMATION FOR SEQ ID NO:56
(i) SEQUENCE CHARACTERISTICS:

- 10 (A) LENGTH: 24
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

- 15 (viii) POSITION IN GENOME:
(B) MAP POSITION: D4S2368

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

TGTACTCATT TTCCCGCAAT GATG

24

- (2) INFORMATION FOR SEQ ID NO:57
(i) SEQUENCE CHARACTERISTICS:

- 20 (A) LENGTH: 24
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

- 25 (viii) POSITION IN GENOME:
(B) MAP POSITION: D4S2368

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

TCAGAAAGTA GGGTCTGGGC TCTT

24

- (2) INFORMATION FOR SEQ ID NO:58
(i) SEQUENCE CHARACTERISTICS:

- 30 (A) LENGTH: 28
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

- 35 (viii) POSITION IN GENOME:
(B) MAP POSITION: D16S539

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

TGTGCATCTG TAAGCATGTA TCTATCAT

28

- (2) INFORMATION FOR SEQ ID NO:59
(i) SEQUENCE CHARACTERISTICS:

- 40 (A) LENGTH: 32

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(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

5 (viii) POSITION IN GENOME:
(B) MAP POSITION: HUMvWFA31

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

GAAAGCCCTA GTGGATGATA AGAATAATCA GT 32

(2) INFORMATION FOR SEQ ID NO:60

10 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

15 (viii) POSITION IN GENOME:
(B) MAP POSITION: HUMvWFA31

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

GGACAGATGA TAAATACATA GGATGGATGG ATA 33

(2) INFORMATION FOR SEQ ID NO:61

20 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

25 (viii) POSITION IN GENOME:
(B) MAP POSITION: D9S930

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:

GCTATGGGAA TTACAAGCAG GAAAC 25

CLAIM(S)

What is claimed is:

1. A method of simultaneously determining the alleles present in at least four short tandem repeat loci from one or more DNA samples, comprising:

a. obtaining at least one DNA sample to be analyzed, wherein the DNA sample has a set of at least four loci which can be amplified together, and wherein the set is selected from the group of loci consisting of:

D3S1539, D4S2368, D5S818, D7S820, D9S930, D10S1239, D13S317, D14S118, D14S548, D14S562, D16S490, D16S539, D16S753, D17S1298, D17S1299, D19S253, D20S481, D22S683, HUMCSF1PO, HUMTPOX, HUMTH01, HUMFESFPS, HUMF13A01, HUMBFXIII, HUMLIPOL, HUMvWFA31;

b. co-amplifying the set of loci in a multiplex amplification reaction, wherein the product of the reaction is a mixture of amplified alleles from each of the co-amplified loci in the set; and

c. evaluating the amplified alleles in the mixture to determine the alleles present at each of the loci analyzed in the set within the DNA sample.

2. The method of claim 1, wherein the set of at least four loci co-amplified therein is a set of four loci, wherein the set of four loci is selected from the group of sets comprising:

D3S1539, D7S820, D13S317, D5S818;
D17S1298, D7S820, D13S317, D5S818;
D20S481, D7S820, D13S317, D5S818;
D9S930, D7S820, D13S317, D5S818;
D10S1239, D7S820, D13S317, D5S818;
D14S118, D7S820, D13S317, D5S818;

D14S562, D7S820, D13S317, D5S818;
D14S548, D7S820, D13S317, D5S818;
D16S490, D7S820, D13S317, D5S818;
D17S1299, D7S820, D13S317, D5S818;
5 D16S539, D7S820, D13S317, D5S818;
D22S683, D7S820, D13S317, D5S818;
D16S753, D7S820, D13S317, D5S818;
D3S1539, D19S253, D13S317, D20S481;
D3S1539, D19S253, D4S2368, D20S481;
10 D10S1239, D9S930, D4S2368, D20S481; and
D16S539, D7S820, D13S317, HUMvWFA31.

3. The method of claim 1, wherein the set of at least four loci co-amplified therein is a set of six loci, wherein the set of six loci is selected from the group of sets comprising:

15 D16S539, D7S820, D13S317, D5S818, HUMCSF1PO, HUMTPOX;
and
D16S539, D7S820, D13S317, D5S818, HUMF13A01, HUMFESFPS.

4. The method of claim 1, wherein the set of at least four loci co-amplified therein is a set of seven loci, wherein the set is selected from
20 the group of sets comprising:

D16S539, D7S820, D13S317, D5S818, HUMCSF1PO, HUMTPOX,
HUMTH01; and
D16S539, D7S820, D13S317, D5S818, HUMF13A01, HUMFESFPS,
HUMBFXIII.

25 5. The method of claim 1, wherein the set of at least four loci co-amplified therein is a set of at least eight loci, and wherein the set is selected from the group of sets comprising:

D16S539, D7S820, D13S317, D5S818, HUMCSF1PO, HUMTPOX, HUMTH01, HUMvWFA31; and

D16S539, D7S820, D13S317, D5S818, HUMF13A01, HUMFESFPS, HUMBFXIII, HUMLIPOL.

5 6. The method of claim 1, wherein the multiplex amplification reaction is done using at least four pair of primers flanking the at least four loci analyzed.

10 7. The method of claim 6, additionally comprising the step of selecting pairs of primers for the multiplex amplification reaction which produce alleles from each locus that do not overlap the alleles of the other loci in the set co-amplified therein, when the alleles are separated by gel electrophoresis.

15 8. The method of claim 6, wherein at least one of each of the pairs of primers used in the multiplex amplification reaction has a sequence selected from one of the groups of sequences consisting of:

SEQ ID NO:1 and SEQ ID NO:2, when one of the loci in the set is D7S820;

SEQ ID NO:3 and SEQ ID NO:4, when one of the loci in the set is D13S317;

20 SEQ ID NO:5 and SEQ ID NO:6, when one of the loci in the set is D5S818;

SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:49, when one of the loci in the set is D3S1539;

25 SEQ ID NO:9, SEQ ID NO:10, when one of the loci in the set is D17S1298;

SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:52, SEQ ID NO: 53, when one of the loci in the set is D20S481;

SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO: 55, SEQ ID NO: 61, when one of the loci in the set is D9S930;

SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:54, when one of the loci in the set is D10S1239;

5 SEQ ID NO:17, SEQ ID NO:18, when one of the loci in the set is D14S118;

SEQ ID NO:19, SEQ ID NO:20, when one of the loci in the set is D14S562;

10 SEQ ID NO:21, SEQ ID NO:22, when one of the loci in the set is D14S548;

SEQ ID NO:23, SEQ ID NO:24, when one of the loci in the set is D16S490;

SEQ ID NO:25, SEQ ID NO:26, when one of the loci in the set is D16S753;

15 SEQ ID NO:27, SEQ ID NO:28, when one of the loci in the set is D17S1299;

SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:58, when one of the loci in the set is D16S539;

20 SEQ ID NO:31, SEQ ID NO:32, when one of the loci in the set is D22S683;

SEQ ID NO:33, SEQ ID NO:34, when one of the loci in the set is HUMCSF1PO;

SEQ ID NO:35, SEQ ID NO:36, when one of the loci in the set is HUMTPOX;

25 SEQ ID NO:37, SEQ ID NO:38, when one of the loci in the set is HUMTH01;

SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:59, SEQ ID NO:60 when one of the loci in the set is HUMvWFA31;

30 SEQ ID NO:41, SEQ ID NO:42, when one of the loci in the set is HUMF13A01;

SEQ ID NO:43, SEQ ID NO:44, when one of the loci in the set is HUMFESFPS;

SEQ ID NO:45, SEQ ID NO:46, when one of the loci in the set is HUMBFXIII;

5 SEQ ID NO:47, SEQ ID NO:48, when one of the loci in the set is HUMLIPOL;

SEQ ID NO:50, SEQ ID NO:51, when one of the loci in the set is D19S253; and

10 SEQ ID NO:56, SEQ ID NO:57, when one of the loci in the set is D4S2368.

9. The method of claim 6, wherein the multiplex amplification reaction is a polymerase chain reaction.

10. The method of claim 1, wherein the amplified alleles are evaluated by comparing the amplified alleles to a size standard, wherein the
15 size standard is selected from the group of size standards consisting of a DNA marker and a locus-specific allelic ladder.

11. The method of claim 1, wherein the amplified alleles are evaluated using polyacrylamide gel electrophoresis to separate the alleles, forming a polyacrylamide gel of separated alleles.

20 12. The method of claim 11, wherein the separated alleles in the polyacrylamide gel are determined by visualizing the alleles with silver stain analysis.

13. The method of claim 11, wherein primers capable of binding to a region flanking each of the loci in the set are used in co-amplifying the
25 loci, wherein at least one of the primers used in co-amplifying each locus has a fluorescent label covalently attached thereto such that the amplified

alleles produced therefrom are fluorescently labeled, and wherein the separated alleles in the polyacrylamide gel are determined by visualizing the alleles with fluorescent analysis.

14. The method of claim 13, wherein the fluorescent label is
5 selected from the group of labels consisting of fluorescein and tetramethyl rhodamine.

15. The method of claim 1 wherein the at least one DNA sample to be analyzed is isolated from human tissue, wherein the human tissue selected from the group of human tissue consisting of blood, semen, vaginal
10 cells, hair, saliva, urine, amniotic fluid containing placental cells or fetal cells, and mixtures of any of the tissues listed above.

16. A method of simultaneously determining the alleles present in three short tandem repeat loci from one or more DNA samples, comprising:
a. obtaining at least one DNA sample to be analyzed, wherein the
15 DNA sample has a set of three short tandem repeat loci which can be amplified together, wherein the set of loci is selected from the following group of sets:

D3S1539, D19S253, D13S317;
D10S1239, D9S930, D20S481;
20 D10S1239, D4S2368, D20S481;
D10S1239, D9S930, D4S2368;
D16S539, D7S820, D13S317; and
D10S1239, D9S930, D13S317.
b. co-amplifying the set of three loci in a multiplex amplification
25 reaction, wherein the product of the reaction is a mixture of amplified alleles from each of the co-amplified loci in the set; and

c. evaluating the amplified alleles in the mixture to determine the alleles present at each of the loci analyzed in the set within the DNA sample.

17. The method of claim 16, wherein the multiplex amplification
5 reaction is done using three pair of primers, wherein each pair of primers flanks one of the three short tandem repeat loci in the set of loci co-amplified in the reaction.

18. The method of claim 17, wherein at least one of the
primers in each pair of primers used in the multiplex amplification reaction
10 has a sequence selected from a group of primer sequences consisting of:

SEQ ID NO:1 and SEQ ID NO:2, when D7S820 is one of the loci in the set of loci co-amplified;

SEQ ID NO:3 and SEQ ID NO:4, when D13S317 is one of the loci in the set of loci co-amplified;

15 SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:52, SEQ ID NO:53, when D20S481 is one of the loci in the set of loci co-amplified;

SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:55 and SEQ ID NO:61, when D9S930 is one of the loci in the set of loci co-amplified;

20 SEQ ID NO:15, SEQ ID NO:16 and SEQ ID NO:44, when D10S1239 is one of the loci in the set of loci co-amplified;

SEQ ID NO:29 and SEQ ID NO:30, when D16S539 is one of the loci in the set of loci co-amplified; and

SEQ ID NO:56 and SEQ ID NO:57, when is one of the loci in the set of loci co-amplified.

25 19. The method of claim 16, wherein the multiplex amplification reaction is a polymerase chain reaction.

20. The method of claim 16, wherein the amplified alleles are evaluated by comparing the amplified alleles to a size standard, wherein the

size standard is selected from the group of size standards consisting of a DNA marker and a locus-specific allelic ladder.

21. The method of claim 16, wherein the amplified alleles are evaluated using polyacrylamide gel electrophoresis to separate the alleles,
5 forming a polyacrylamide gel of separated alleles.

22. The method of claim 21, wherein the separated alleles in the polyacrylamide gel are determined by visualizing the alleles with silver stain analysis.

23. The method of claim 21, wherein the separated alleles in the
10 polyacrylamide gel are determined by visualizing the alleles with fluorescent analysis.

24. The method of claim 16 wherein the at least one DNA sample to be analyzed is isolated from human tissue, wherein the human tissue selected from the group of human tissue consisting of blood, semen, vaginal
15 cells, hair, saliva, urine, bone, buccal sample, amniotic fluid containing placental cells or fetal cells, and mixtures of any of the tissues listed above.

25. A kit for simultaneously analyzing short tandem repeat sequences in at least three loci, comprising a container which has oligonucleotide primers for co-amplifying a set of at least three short tandem
20 repeat loci, wherein the set of loci are selected from the sets of loci consisting of:

D3S1539, D19S253, D13S317;

D10S1239, D9S930, D20S481;

D10S1239, D4S2368, D20S481;

25 D10S1239, D9S930, D4S2368;

D16S539, D7S820, D13S317;

D10S1239, D9S930, D13S317;
D3S1539, D7S820, D13S317, D5S818;
D17S1298, D7S820, D13S317, D5S818;
D20S481, D7S820, D13S317, D5S818;
5 D9S930, D7S820, D13S317, D5S818;
D10S1239, D7S820, D13S317, D5S818;
D14S118, D7S820, D13S317, D5S818;
D14S562, D7S820, D13S317, D5S818;
D14S548, D7S820, D13S317, D5S818;
10 D16S490, D7S820, D13S317, D5S818;
D17S1299, D7S820, D13S317, D5S818;
D16S539, D7S820, D13S317, D5S818;
D22S683, D7S820, D13S317, D5S818;
D16S753, D7S820, D13S317, D5S818;
15 D3S1539, D19S253, D13S317, D20S481;
D3S1539, D19S253, D4S2368, D20S481;
D10S1239, D9S930, D4S2368, D20S481;
D16S539, D7S820, D13S317, HUMvWFA31;
D16S539, D7S820, D13S317, D5S818, HUMCSF1PO, HUMTPOX;
20 D16S539, D7S820, D13S317, D5S818, HUMF13A01, HUMFESFPS;
D16S539, D7S820, D13S317, D5S818, HUMCSF1PO, HUMTPOX,
HUMTH01;
D16S539, D7S820, D13S317, D5S818, HUMF13A01, HUMFESFPS,
HUMBFXIII;
25 D16S539, D7S820, D13S317, D5S818, HUMCSF1PO, HUMTPOX,
HUMTH01, HUMvWFA31; and
D16S539, D7S820, D13S317, D5S818, HUMF13A01, HUMFESFPS,
HUMBFXIII, HUMLIPOL.

26. The kit of claim 25, at least one of the oligonucleotide primers for each of the loci in the set has a sequence selected from a group of sequences consisting of:

5 SEQ ID NO:1 and SEQ ID NO:2, when D7S820 is one of the loci in the set;

SEQ ID NO:3 and SEQ ID NO:4, when D13S317 is one of the loci in the set;

SEQ ID NO:5 and SEQ ID NO:6, when one of the loci in the set is D5S818;

10 SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:49, when one of the loci in the set is D3S153;

SEQ ID NO:9, SEQ ID NO:10, when one of the loci in the set is D17S1298;

15 SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:52, SEQ ID NO: 53, when one of the loci in the set is D20S481;

SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO: 55, SEQ ID NO: 61, when one of the loci in the set is D9S930;

SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:54, when one of the loci in the set is D10S1239;

20 SEQ ID NO:17, SEQ ID NO:18, when one of the loci in the set is D14S118;

SEQ ID NO:19, SEQ ID NO:20, when one of the loci in the set is D14S562;

25 SEQ ID NO:21, SEQ ID NO:22, when one of the loci in the set is D14S548;

SEQ ID NO:23, SEQ ID NO:24, when one of the loci in the set is D16S490;

SEQ ID NO:25, SEQ ID NO:26, when one of the loci in the set is D16S753;

30 SEQ ID NO:27, SEQ ID NO:28, when one of the loci in the set is D17S1299;

SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:58, when one of the loci in the set is D16S539;

SEQ ID NO:31, SEQ ID NO:32, when one of the loci in the set is D22S683;

5 SEQ ID NO:33, SEQ ID NO:34, when one of the loci in the set is HUMCSF1PO;

SEQ ID NO:35, SEQ ID NO:36, when one of the loci in the set is HUMTPOX;

10 SEQ ID NO:37, SEQ ID NO:38, when one of the loci in the set is HUMTH01;

SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:60 when one of the loci in the set is HUMvWFA31;

SEQ ID NO:41, SEQ ID NO:42, when one of the loci in the set is HUMF13A01;

15 SEQ ID NO:43, SEQ ID NO:44, when one of the loci in the set is HUMFESFPS;

SEQ ID NO:45, SEQ ID NO:46, when one of the loci in the set is HUMBFXIII;

20 SEQ ID NO:47, SEQ ID NO:48, when one of the loci in the set is HUMLIPOL;

SEQ ID NO:50, SEQ ID NO:51, when one of the loci in the set is D19S253; and

SEQ ID NO:56, SEQ ID NO:57, when one of the loci in the set is D4S2368.

25 27. The kit of claim 25, further comprising a container having reagents for at least one multiplex amplification reaction.

28. The kit of claim 25, further comprising a container having an allelic ladder.

29. The kit of claim 28, wherein each rung of the allelic ladder and at least one oligonucleotide primer for each of the loci in the set each have a label covalently attached thereto.

30. The kit of claim 29, wherein the label is a fluorescent label.

5 31. The kit of claim 30, wherein at least one of the oligonucleotide primers has a different fluorescent label covalently attached thereto than some of the other primer pairs in the container.

32. A pair of oligonucleotide primers flanking locus D3S1539 in human DNA, wherein one primer of the pair has a sequence of SEQ ID
10 NO:7, and wherein the other primer of the pair has a sequence selected from the group consisting of SEQ ID NO:8 and SEQ ID NO:49.

33. A pair of oligonucleotide primers flanking locus D17S1298 in human DNA, wherein one primer of the pair has a sequence of SEQ ID
15 NO:9, and wherein the other primer of the pair has a sequence of SEQ ID NO:10.

34. A pair of oligonucleotide primers flanking locus D20S481 in human DNA, wherein one primer of the pair has a sequence selected from the group consisting of SEQ ID NO:11 and SEQ ID NO:52, and wherein the
20 other primer of the pair has a sequence selected from the group consisting of SEQ ID NO:12 and SEQ ID NO:53.

35. A pair of oligonucleotide primers flanking locus D9S930 in human DNA, wherein one primer of the pair has a sequence of SEQ ID NO:55, and wherein the other primer of the pair has a sequence selected from the group consisting of SEQ ID NO:14 and SEQ ID NO:61.

36. A pair of oligonucleotide primers flanking locus D4S2368 in human DNA, wherein one primer of the pair has a sequence of SEQ ID NO:56 and wherein the other primer of the pair has a sequence of SEQ ID NO:57.

5 37. A method of simultaneously determining the alleles present in at least four short tandem repeat loci from one or more DNA samples, comprising:

- 10 a. obtaining at least one DNA sample to be analyzed, wherein the DNA sample has a set of at least four loci which can be amplified together, and wherein three of the loci in the set are D7S820, D13S317, and D5S818;
- b. co-amplifying the set of loci in a multiplex amplification reaction, wherein the product of the reaction is a mixture of amplified alleles from each of the co-amplified loci in the set; and
- 15 c. evaluating the amplified alleles in the mixture to determine the alleles present at each of the loci analyzed in the set within the DNA sample.

38. The method of claim 37, wherein the multiplex amplification reaction is done using at least four pair of primers flanking the
20 at least four loci analyzed.

39. The method of claim 38, additionally comprising the step of selecting pairs of primers for the multiplex amplification reaction which produce alleles from each locus that do not overlap the alleles of the other loci in the set co-amplified therein, when the alleles are separated by gel
25 electrophoresis.

40. The method of claim 37, wherein the multiplex amplification reaction is a polymerase chain reaction.

41. The method of claim 37, wherein the amplified alleles are evaluated by comparing the amplified alleles to a size standard, wherein the size standard is selected from the group of size standards consisting of a DNA marker and a locus-specific allelic ladder.

5 42. The method of claim 37, wherein the amplified alleles are evaluated using polyacrylamide gel electrophoresis to separate the alleles, forming a polyacrylamide gel of separated alleles.

 43. The method of claim 42, wherein the separated alleles in the polyacrylamide gel are determined by visualizing the alleles with silver stain
10 analysis.

 44. The method of claim 42, wherein primers capable of binding to a region flanking each of the loci in the set are used in co-amplifying the loci, wherein at least one of the primers used in co-amplifying each locus has a fluorescent label covalently attached thereto such that the amplified
15 alleles produced therefrom are fluorescently labeled, and wherein the separated alleles in the polyacrylamide gel are determined by visualizing the alleles with fluorescent analysis.

 45. The method of claim 44, wherein the fluorescent label is selected from the group of labels consisting of fluorescein and tetramethyl
20 rhodamine.

1/19



FIGURE 1

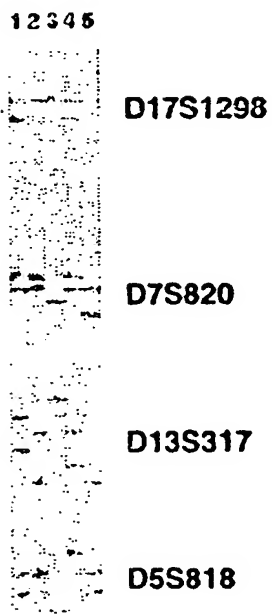


FIGURE 2

2/19

1 2 3 4 5

D17S1298

D7S820

D13S317

D5S818

FIGURE 3

1 2 3 4 5

D20S481

D7S820

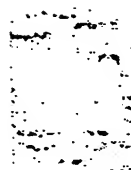
D13S317

D5S818

FIGURE 4

3/19

1 2 3 4 5



D20S481



D7S820



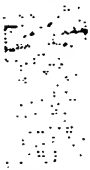
D13S317



D5S818

FIGURE 5

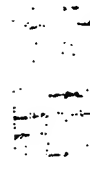
1 2 3 4 5



D9S930



D7S820



D13S317



D5S818

FIGURE 6

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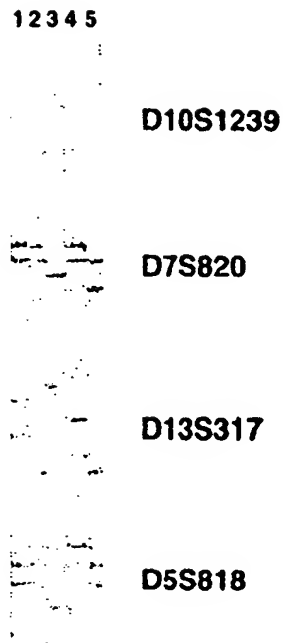


FIGURE 7

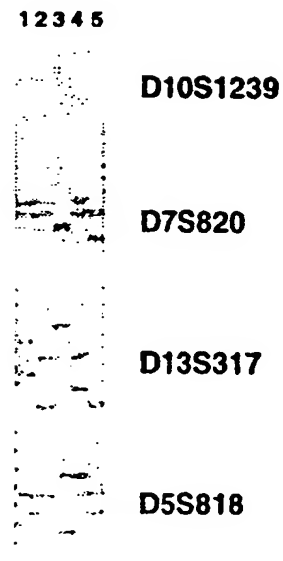


FIGURE 8

5/19



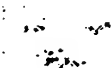
FIGURE 9



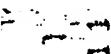
FIGURE 10

6/19

1 2 3 4 5



D14S548



D7S820



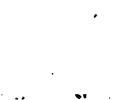
D13S317



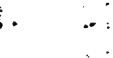
D5S818

FIGURE 11

1 2 3 4 5



D16S490



D7S820



D13S317



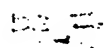
D5S818

FIGURE 12

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1 2 3 4 5

 D16S753

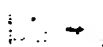
 D7S820

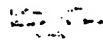
 D13S317

 D5S818

FIGURE 13

1 2 3 4 5

 D17S1299

 D7S820

 D13S317

 D5S818

FIGURE 14

8/19

1 2 3 4 5

D16S539

D7S820

D13S317

D5S818

FIGURE 15

1 2 3 4 5

D22S683

D7S820

D13S317

D5S818

FIGURE 16

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FIGURE 17

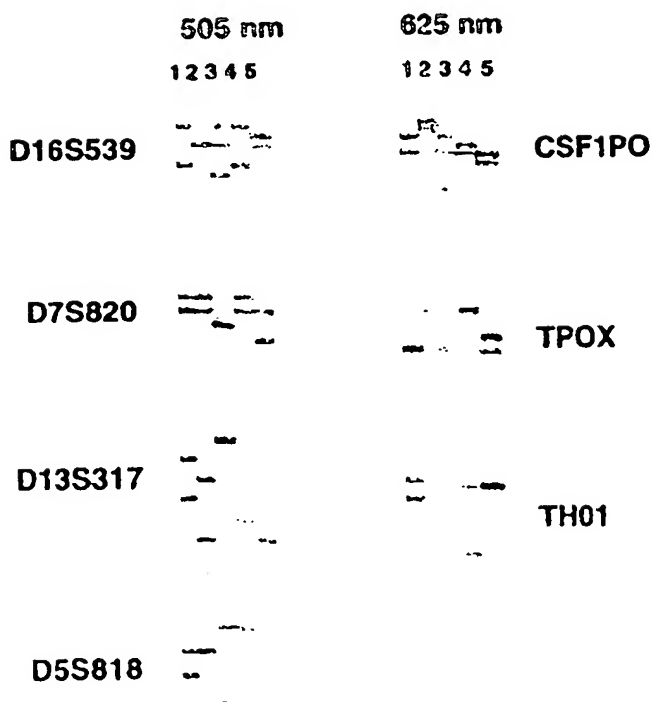


FIGURE 18

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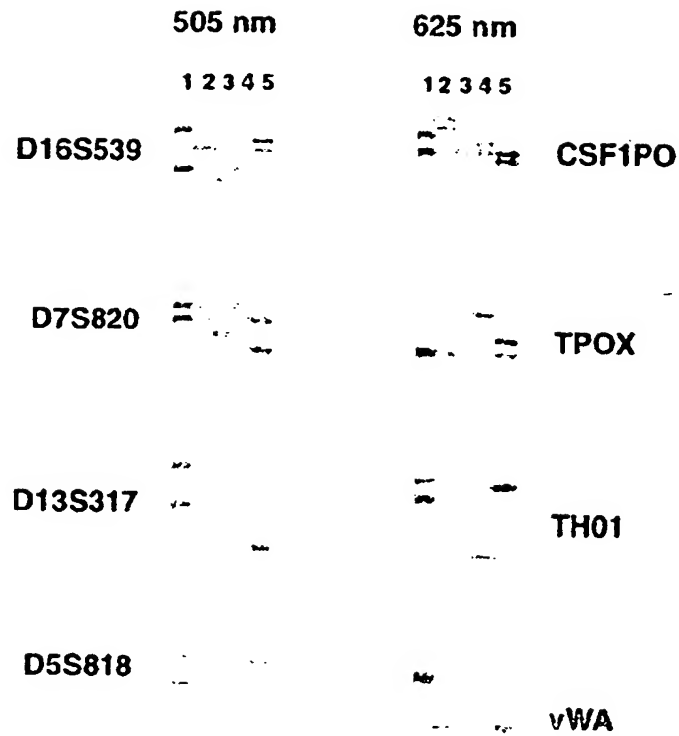


FIGURE 19

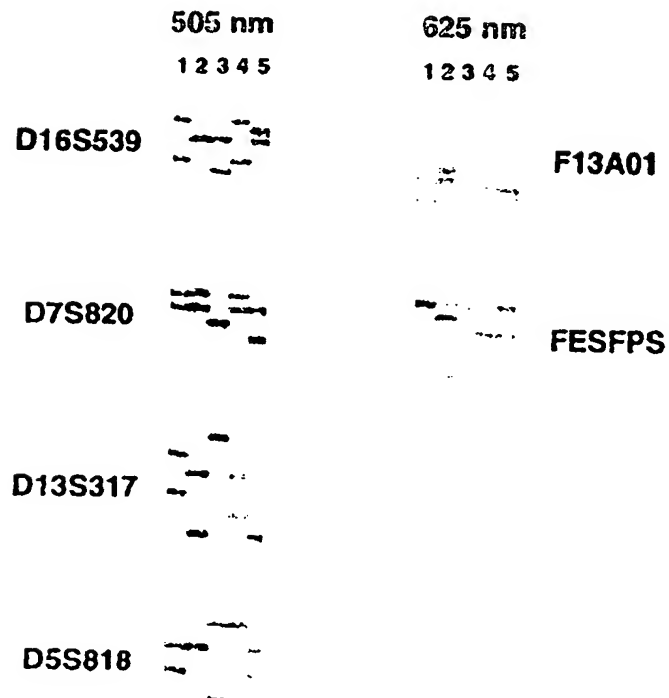


FIGURE 20

11/19

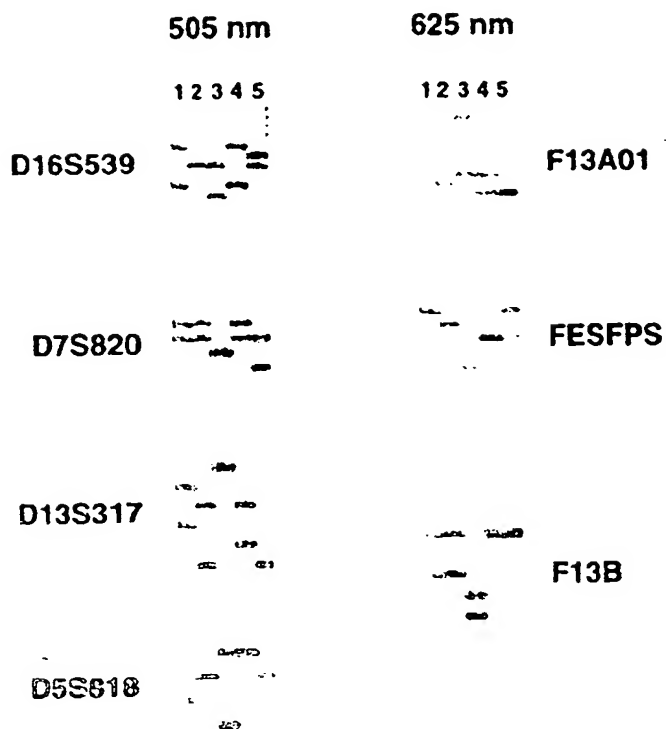


FIGURE 21

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FIGURE 22

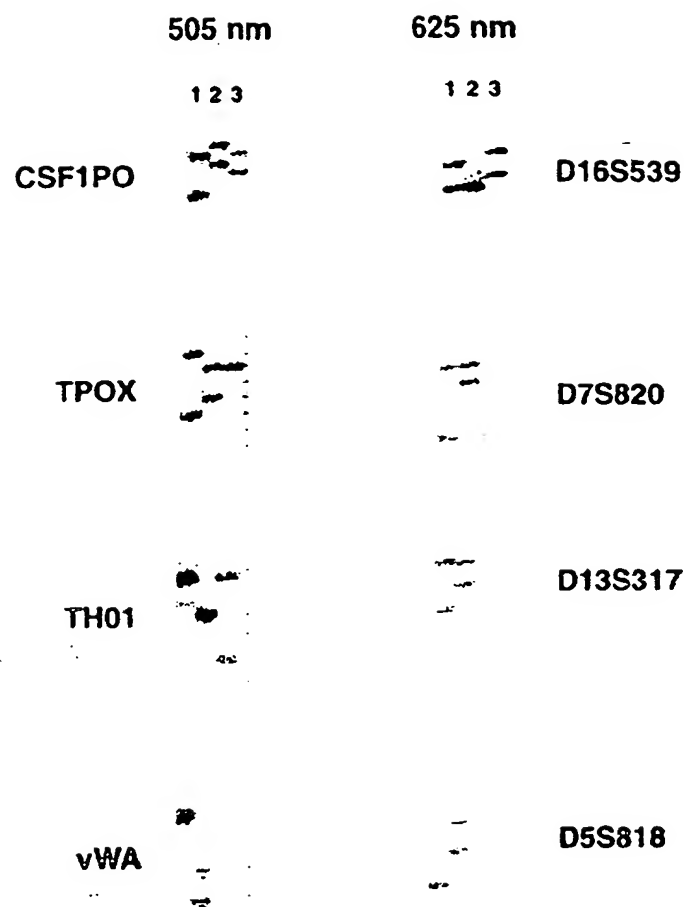


FIGURE 23

14/19

1 2 3 4 5



D3S1539



D19S253



D13S317



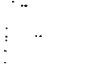
D20S481

FIGURE 24

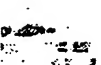
1 2 3 4 5



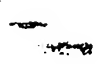
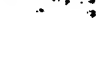
D10S1239



D9S930



D4S2368



D20S481

FIGURE 25

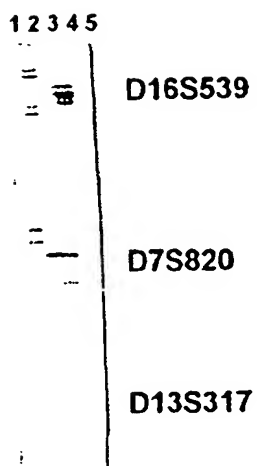


FIGURE 26

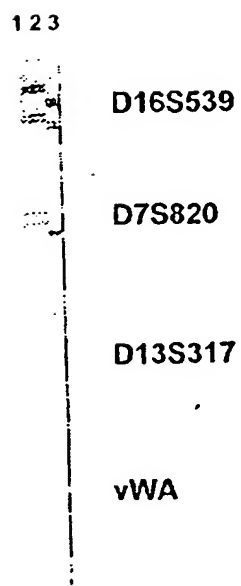


FIGURE 27

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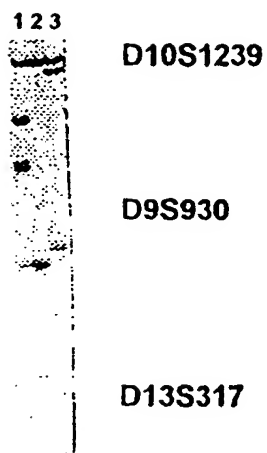


FIGURE 28

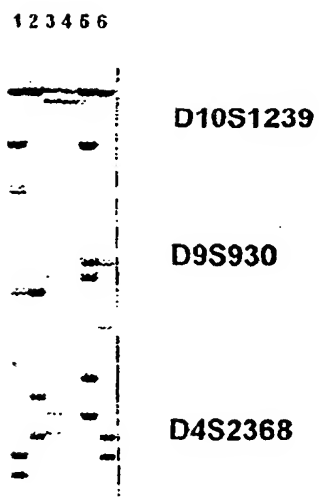


FIGURE 29

SUBSTITUTE SHEET (RULE 26)

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FIGURE 30

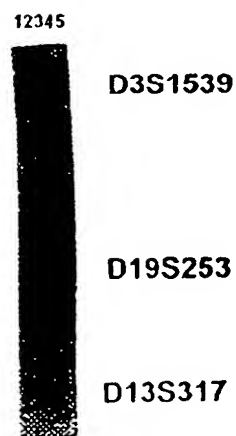


FIGURE 31

SUBSTITUTE SHEET (RULE 26)

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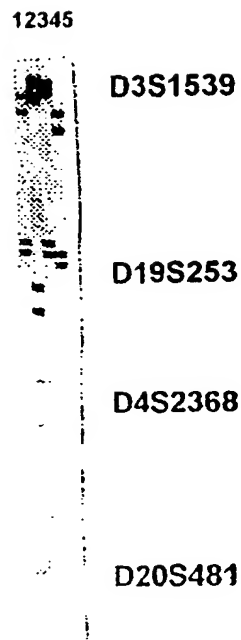


FIGURE 32

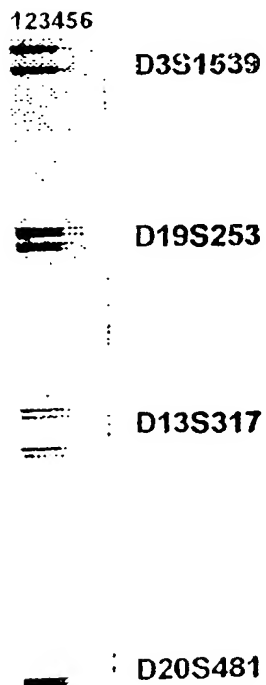


FIGURE 33

SUBSTITUTE SHEET (RULE 26)

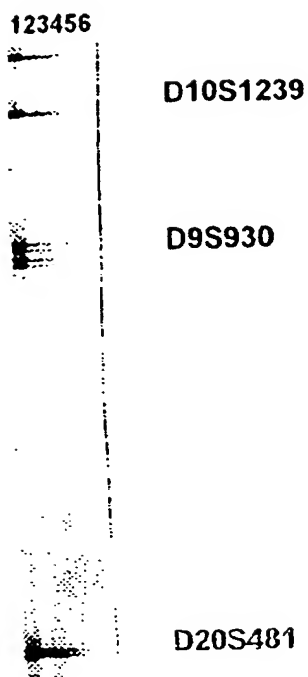


FIGURE 34



FIGURE 35

SUBSTITUTE SHEET (RULE 26)

INTERNATIONAL SEARCH REPORT

International application No
PCT/US97/06293

A. CLASSIFICATION OF SUBJECT MATTER IPC(6) : C12P 19/34; C12Q 1/68; C07H 21/04 US CL : 435/91.2, 6, 810; 536/24.3, 24.33 According to International Patent Classification (IPC) or to both national classification and IPC																									
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) U.S. : 435/91.2, 6, 810; 536/24.3, 24.33 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) Please See Extra Sheet.																									
C. DOCUMENTS CONSIDERED TO BE RELEVANT																									
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.																							
X --- Y	URQUHART et al. Variation in Short Tandem Repeat sequences - a survey of twelve microsatellite loci for use as forensic identification markers. Int. J. Legal Med. 1994. Vol. 107. Pages 13-20, see entire document.	1, 2, 6-11, 15-17, 19-21, 24-28, 37-42 ----- 12, 22, 43																							
X	KIMPTON et al. Evaluation of an automated DNA profiling system employing multiplex amplification of four tetrameric STR loci. Int. J. Legal Med. 1994. Vol. 106. Pages 302-311, see pages 302-303.	1, 2, 6-11, 13-17, 19-21, 23-27, 37-42, 44, 45																							
X	TULLY et al. Analysis of 6 VNTR loci by 'multiplex' PCR and automated fluorescent detection. Human Genetics. 1993. Vol. 92. Pages 554-562, see pages 554-555.	1, 3, 16, 17-21																							
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/06293

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KIMPTON et al. Automated DNA Profiling Employing Multiplex Amplification of Short Tandem Repeat Loci. Adv. Forensic Haemogenetics. 1994. Vol. 5. Pages 309-311, see entire document.	25, 27-31
X	MCKEOWN et al. Increasing the size of PCR products without redesigning primer binding sequences. Nucleic Acids Research. November 1995. Vol. 23. No. 12. Pages 2337-2338, see entire document.	1, 3-5
X	OLDROYD et al. A highly discriminating octoplex short tandem repeat polymerase chain reaction system suitable for human individual identification. Electrophoresis. 1995. Vol. 16. Pages 334-337, see page 335.	1, 3, 4
X	OETTING et al. Linkage Analysis with Multiplexed Short Tandem Repeat Polymorphisms Using Infrared Fluorescence and M13 Tailed Primers. Genomics. 1995. Vol. 30. Pages 450-458, see page 451, 454	1, 2, 6, 9-13, 15-17, 19-21, 23, 24, 37, 38, 40-42, 44
X --- Y	LIN et al. Multiplex Genotype Determination at a Large Number of Gene Loci. Proc. Natl. Acad. Sci. USA. March 1996. Vol. 93. Pages 2582-2587, see abstract and page 2584.	1, 3-6, 9, 11, 16, 19, 21, 16, 37, 38, 40, 42 ----- 12, 22, 43
X	FUENTES et al. Genetic Variation of Microsatellite Markers DIS117, D6S89, D11S35, APOC2, and D21S168 in the Spanish Population. Int. J. Legal Med. 1993. Vol. 105. Pages 271-277, see page 275.	1, 6, 7, 9-11, 13-17, 19-21, 23, 24, 37-42, 44, 45
Y	BASSAM et al. Fast and Sensitive Silver Staining of DNA in Polyacrylamide Gels. Analytical Biochemistry. 1991. Vol. 196. Pages 80-83, see entire document.	12, 22, 43
X	GERKEN et al. GENBANK ACCESSION NUMBER L16393. May 1993.	32
X	MURRAY et al. GENBANK ACCESSION NUMBER G07948. August 1995.	33
X	MURRAY et al. GENBANK ACCESSION NUMBER G08051. August 1995.	34
X	MURRAY et al. GENBANK ACCESSION NUMBER G08745. August 1995.	35

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/06293

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Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MURRAY et al. GENBANK ACCESSION NUMBER G08343. August 1995.	36

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C12P 19/34, C12Q 1/68, C07H 21/04		A1	(11) International Publication Number: WO 97/39138
			(43) International Publication Date: 23 October 1997 (23.10.97)
(21) International Application Number: PCT/US97/06293		(81) Designated States: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TT, UA, UG, UZ, VN, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).	
(22) International Filing Date: 15 April 1997 (15.04.97)			
(30) Priority Data: 08/632,575 15 April 1996 (15.04.96) US			
(71) Applicant: PROMEGA CORPORATION [US/US]; 2800 Woods Hollow Road, Madison, WI 53711-5399 (US).			
(72) Inventors: SCHUMM, James, W.; 5843 Timber Ridge Trail, Madison, WI 53711 (US). MICKA, Katherine, A.; 1842 Paddock Place, Oregon, WI 53575 (US). RABBACH, Dawn, R.; 6649 Wendell Way, DeForest, WI 53532 (US).		Published With international search report. With amended claims and statement.	
(74) Agents: FRENCHICK, Grady, J. et al.; Stroud, Stroud, Willink, Thompson & Howard, Suite 300, 25 West Main Street, P.O. Box 2236, Madison, WI 53701-2236 (US).		Date of publication of the amended claims and statement: 31 December 1997 (31.12.97)	
(54) Title: MULTIPLEX AMPLIFICATION OF SHORT TANDEM REPEAT LOCI			
(57) Abstract <p>The invention is directed to the simultaneous amplification of multiple distinct genetic loci using PCR or other amplification systems to determine in one multiplex reaction the alleles of each of the loci contained in the multiplex reaction. The genetic loci analyzed comprise the following: HUMvWFA31, HUMLIPOL, HUMBFXIII, HUMF13A01, HUMFESFPS, HUMTH01, HUMTPOX, HUMCSF1PO, D22S683, D20S481, D19S253, D17S1299, D17S1298, D16S753, D16S539, D16S490, D14S562, D14S548, D14S118, D13S317, D10S1239, D9S930, D7S820, D5S818, D4S2368, D3S1539.</p>			

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AMENDED CLAIMS

[received by the International Bureau on 21 November 1997 (21.11.97);
original claims 1-5, 16, 18, 26 and 37 amended; remaining claims unchanged (15 pages)]

1. A method of simultaneously determining the alleles present in
at least four short tandem repeat loci from one or more DNA samples,
5 comprising:

- a. obtaining at least one DNA sample to be analyzed,
- b. selecting a set of at least four short tandem repeat loci of the
DNA sample to be analyzed which can be amplified together, wherein
at least four of the loci in the set are selected from the group of loci
10 consisting of:

D3S1539, D4S2368, D5S818, D7S820, D9S930, D10S1239,
D13S317, D14S118, D14S548, D14S562, D16S490,
D16S539, D16S753, D17S1298, D17S1299, D19S253,
D20S481, D22S683, HUMCSF1PO, HUMTPOX, HUMTH01,
15 HUMFESFPS, HUMF13A01, HUMBFXIII, HUMLIPOI,
HUMvWFA31;

- c. co-amplifying the set of loci in a multiplex amplification reaction,
wherein the product of the reaction is a mixture of amplified alleles
from each of the co-amplified loci in the set; and
- 20 d. evaluating the amplified alleles in the mixture to determine the
alleles present at each of the loci analyzed in the set within the DNA
sample.

2. The method of claim 1, wherein the set of at least four loci
25 co-amplified therein is a set of four loci, wherein the set of four loci is
selected from the group of sets of loci consisting of:

D3S1539, D7S820, D13S317, D5S818;
D17S1298, D7S820, D13S317, D5S818;
D20S481, D7S820, D13S317, D5S818;
30 D9S930, D7S820, D13S317, D5S818;
D10S1239, D7S820, D13S317, D5S818;

5 D14S118, D7S820, D13S317, D5S818;
D14S562, D7S820, D13S317, D5S818;
D14S548, D7S820, D13S317, D5S818;
D16S490, D7S820, D13S317, D5S818;
D17S1299, D7S820, D13S317, D5S818;
D16S539, D7S820, D13S317, D5S818;
D22S683, D7S820, D13S317, D5S818;
D16S753, D7S820, D13S317, D5S818;
D3S1539, D19S253, D13S317, D20S481;
10 D3S1539, D19S253, D4S2368, D20S481;
D10S1239, D9S930, D4S2368, D20S481; and
D16S539, D7S820, D13S317, HUMvWFA31.

15 3. The method of claim 1, wherein the set of at least four loci co-amplified therein is a set of six loci, wherein the set of six loci is selected from the group of sets of loci consisting of:

D16S539, D7S820, D13S317, D5S818, HUMCSF1PO, HUMTPOX; and
D16S539, D7S820, D13S317, D5S818, HUMF13A01, HUMFESFPS.

20 4. The method of claim 1, wherein the set of at least four loci co-amplified therein is a set of seven loci, wherein the set is selected from the group of sets of loci consisting of:

D16S539, D7S820, D13S317, D5S818, HUMCSF1PO, HUMTPOX,
HUMTH01; and

25 D16S539, D7S820, D13S317, D5S818, HUMF13A01, HUMFESFPS,
HUMBFXIII.

30 5. The method of claim 1, wherein the set of at least four loci co-amplified therein is a set of at least eight loci, and wherein the set is selected from the group of sets of loci consisting of:

D16S539, D7S820, D13S317, D5S818, HUMCSF1PO, HUMTPOX, HUMTH01, HUMvWFA31; and

D16S539, D7S820, D13S317, D5S818, HUMF13A01, HUMFESFPS, HUMBFXIII, HUMLIPOL.

5

6. The method of claim 1, wherein the multiplex amplification reaction is done using at least four pair of primers flanking the at least four loci analyzed.

10

7. The method of claim 6, additionally comprising the step of selecting pairs of primers for the multiplex amplification reaction which produce alleles from each locus that do not overlap the alleles of the other loci in the set co-amplified therein, when the alleles are separated by gel electrophoresis.

15

8. The method of claim 6, wherein at least one of each of the pairs of primers used in the multiplex amplification reaction has a sequence selected from one of the groups of sequences consisting of:

SEQ ID NO:1 and SEQ ID NO:2, when one of the loci in the set is
20 D7S820;

SEQ ID NO:3 and SEQ ID NO:4, when one of the loci in the set is
D13S317;

SEQ ID NO:5 and SEQ ID NO:6, when one of the loci in the set is
D5S818;

25 SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:49, when one of the loci in the set is D3S1539;

SEQ ID NO:9, SEQ ID NO:10, when one of the loci in the set is
D17S1298;

30 SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:52, SEQ ID NO: 53, when one of the loci in the set is D20S481;

SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO: 55, SEQ ID NO: 61, when one of the loci in the set is D9S930;

SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:54, when one of the loci in the set is D10S1239;

5 SEQ ID NO:17, SEQ ID NO:18, when one of the loci in the set is D14S118;

SEQ ID NO:19, SEQ ID NO:20, when one of the loci in the set is D14S562;

10 SEQ ID NO:21, SEQ ID NO:22, when one of the loci in the set is D14S548;

SEQ ID NO:23, SEQ ID NO:24, when one of the loci in the set is D16S490;

SEQ ID NO:25, SEQ ID NO:26, when one of the loci in the set is D16S753;

15 SEQ ID NO:27, SEQ ID NO:28, when one of the loci in the set is D17S1299;

SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:58, when one of the loci in the set is D16S539;

20 SEQ ID NO:31, SEQ ID NO:32, when one of the loci in the set is D22S683;

SEQ ID NO:33, SEQ ID NO:34, when one of the loci in the set is HUMCSF1PO;

SEQ ID NO:35, SEQ ID NO:36, when one of the loci in the set is HUMTPOX;

25 SEQ ID NO:37, SEQ ID NO:38, when one of the loci in the set is HUMTH01;

SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:59, SEQ ID NO:60 when one of the loci in the set is HUMvWFA31;

30 SEQ ID NO:41, SEQ ID NO:42, when one of the loci in the set is HUMF13A01;

AMENDED SHEET (ARTICLE 19)

SEQ ID NO:43, SEQ ID NO:44, when one of the loci in the set is HUMFESFPS;

SEQ ID NO:45, SEQ ID NO:46, when one of the loci in the set is HUMBFXIII;

5 SEQ ID NO:47, SEQ ID NO:48, when one of the loci in the set is HUMLIPOL;

SEQ ID NO:50, SEQ ID NO:51, when one of the loci in the set is D19S253; and

10 SEQ ID NO:56, SEQ ID NO:57, when one of the loci in the set is D4S2368.

9. The method of claim 6, wherein the multiplex amplification reaction is a polymerase chain reaction.

15 10. The method of claim 1, wherein the amplified alleles are evaluated by comparing the amplified alleles to a size standard, wherein the size standard is selected from the group of size standards consisting of a DNA marker and a locus-specific allelic ladder.

20 11. The method of claim 1, wherein the amplified alleles are evaluated using polyacrylamide gel electrophoresis to separate the alleles, forming a polyacrylamide gel of separated alleles.

25 12. The method of claim 11, wherein the separated alleles in the polyacrylamide gel are determined by visualizing the alleles with silver stain analysis.

30 13. The method of claim 11, wherein primers capable of binding to a region flanking each of the loci in the set are used in co-amplifying the loci, wherein at least one of the primers used in co-amplifying each locus has a fluorescent label covalently attached thereto such that the amplified alleles

produced therefrom are fluorescently labeled, and wherein the separated alleles in the polyacrylamide gel are determined by visualizing the alleles with fluorescent analysis.

5 14. The method of claim 13, wherein the fluorescent label is selected from the group of labels consisting of fluorescein and tetramethyl rhodamine.

10 15. The method of claim 1 wherein the at least one DNA sample to be analyzed is isolated from human tissue, wherein the human tissue selected from the group of human tissue consisting of blood, semen, vaginal cells, hair, saliva, urine, amniotic fluid containing placental cells or fetal cells, and mixtures of any of the tissues listed above.

15 16. A method of simultaneously determining the alleles present in three short tandem repeat loci from one or more DNA samples, comprising:

- 20 a. obtaining at least one DNA sample to be analyzed,
 b. selecting a set of three short tandem repeat loci of the DNA sample to be analyzed which can be amplified together, wherein the set of three loci is selected from the group of sets of loci consisting of:

D3S1539, D19S253, D13S317;

D10S1239, D9S930, D20S481;

D10S1239, D4S2368, D20S481;

25 D10S1239, D9S930, D4S2368;

D16S539, D7S820, D13S317; and

D10S1239, D9S930, D13S317.

- 30 c. co-amplifying the set of three loci in a multiplex amplification reaction, wherein the product of the reaction is a mixture of amplified alleles from each of the co-amplified loci in the set; and

d. evaluating the amplified alleles in the mixture to determine the alleles present at each of the loci analyzed in the set within the DNA sample.

5 17. The method of claim 16, wherein the multiplex amplification reaction is done using three pair of primers, wherein each pair of primers flanks one of the three short tandem repeat loci in the set of loci co-amplified in the reaction.

10 18. The method of claim 17, wherein each of the three pair of primers used in the multiplex amplification reaction is designed to hybridize with an allele of a locus in the set of loci co-amplified in the reaction wherein:

 when D7S820 is one of the loci in the set of loci co-amplified, at least
15 one of the primers has a sequence selected from the group consisting of SEQ ID NO:1 and SEQ ID NO:2;

 when D13S317 is one of the loci in the set of loci co-amplified, at least one of the primers has a sequence selected from the group consisting of SEQ ID NO:3 and SEQ ID NO:4;

20 when D20S481 is one of the loci in the set of loci co-amplified, at least one of the primers has a sequence selected from the group consisting of SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:52, SEQ ID NO:53;

 when D9S930 is one of the loci in the set of loci co-amplified, at least one of the primers has a sequence selected from the group consisting of SEQ
25 ID NO:13, SEQ ID NO:14, SEQ ID NO:55 and SEQ ID NO:61;

 when D10S1239 is one of the loci in the set of loci co-amplified, at least one of the primers has a sequence selected from the group consisting of SEQ ID NO:15, SEQ ID NO:16 and SEQ ID NO:44;

30 when D16S539 is one of the loci in the set of loci co-amplified, at least one of the primers has a sequence selected from the group consisting of SEQ ID NO:29 and SEQ ID NO:30; and

when D4S2368 is one of the loci in the set of loci co-amplified, at least one of the primers has a sequence selected from the group consisting of SEQ ID NO:56 and SEQ ID NO:57.

5

19. The method of claim 16, wherein the multiplex amplification reaction is a polymerase chain reaction.

10

20. The method of claim 16, wherein the amplified alleles are evaluated by comparing the amplified alleles to a size standard, wherein the size standard is selected from the group of size standards consisting of a DNA marker and a locus-specific allelic ladder.

15

21. The method of claim 16, wherein the amplified alleles are evaluated using polyacrylamide gel electrophoresis to separate the alleles, forming a polyacrylamide gel of separated alleles.

20

22. The method of claim 21, wherein the separated alleles in the polyacrylamide gel are determined by visualizing the alleles with silver stain analysis.

25

23. The method of claim 21, wherein the separated alleles in the polyacrylamide gel are determined by visualizing the alleles with fluorescent analysis.

30

24. The method of claim 16 wherein the at least one DNA sample to be analyzed is isolated from human tissue, wherein the human tissue selected from the group of human tissue consisting of blood, semen, vaginal cells, hair, saliva, urine, bone, buccal sample, amniotic fluid containing placental cells or fetal cells, and mixtures of any of the tissues listed above.

25. A kit for simultaneously analyzing short tandem repeat sequences in at least three loci, comprising a container which has oligonucleotide primers for co-amplifying a set of at least three short tandem repeat loci, wherein the set of loci are selected from the sets of loci consisting of:

- 5 D3S1539, D19S253, D13S317;
 D10S1239, D9S930, D20S481;
 D10S1239, D4S2368, D20S481;
 D10S1239, D9S930, D4S2368;
10 D16S539, D7S820, D13S317;
 D10S1239, D9S930, D13S317;
 D3S1539, D7S820, D13S317, D5S818;
 D17S1298, D7S820, D13S317, D5S818;
 D20S481, D7S820, D13S317, D5S818;
15 D9S930, D7S820, D13S317, D5S818;
 D10S1239, D7S820, D13S317, D5S818;
 D14S118, D7S820, D13S317, D5S818;
 D14S562, D7S820, D13S317, D5S818;
 D14S548, D7S820, D13S317, D5S818;
20 D16S490, D7S820, D13S317, D5S818;
 D17S1299, D7S820, D13S317, D5S818;
 D16S539, D7S820, D13S317, D5S818;
 D22S683, D7S820, D13S317, D5S818;
 D16S753, D7S820, D13S317, D5S818;
25 D3S1539, D19S253, D13S317, D20S481;
 D3S1539, D19S253, D4S2368, D20S481;
 D10S1239, D9S930, D4S2368, D20S481;
 D16S539, D7S820, D13S317, HUMvWFA31;
 D16S539, D7S820, D13S317, D5S818, HUMCSF1PO, HUMTPOX;
30 D16S539, D7S820, D13S317, D5S818, HUMF13A01, HUMFESFPS;

D16S539, D7S820, D13S317, D5S818, HUMCSF1PO, HUMTPOX, HUMTH01;

D16S539, D7S820, D13S317, D5S818, HUMF13A01, HUMFESFPS, HUMBFXIII;

5 D16S539, D7S820, D13S317, D5S818, HUMCSF1PO, HUMTPOX, HUMTH01, HUMvWFA31; and

D16S539, D7S820, D13S317, D5S818, HUMF13A01, HUMFESFPS, HUMBFXIII, HUMLIPOL.

10 26. The kit of claim 25, wherein each of the oligonucleotide primers is designed to hybridize with an allele of one of the three loci in the set of loci selected, wherein:

when D7S820 is one of the loci in the set, at least one of the primers has a sequence selected from the group consisting of SEQ ID NO:1 and SEQ
15 ID NO:2;

when D13S317 is one of the loci in the set, at least one of the primers has a sequence selected from the group consisting of SEQ ID NO:3 and SEQ
ID NO:4;

when D5S818, at least one of the primers has a sequence selected
20 from the group consisting of SEQ ID NO:5 and SEQ ID NO:6;

when D3S153, at least one of the primers has a sequence selected from the group consisting of SEQ ID NO:7, SEQ ID NO:8 and SEQ ID NO:49;

when D17S1298, at least one of the primers has a sequence selected from the group consisting of SEQ ID NO:9 and SEQ ID NO:10;

25 when D20S481, at least one of the primers has a sequence selected from the group consisting of SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:52, SEQ ID NO:53;

when D9S930, at least one of the primers has a sequence selected from the group consisting of SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:55,
30 SEQ ID NO:61;

- when D10S1239, at least one of the primers has a sequence selected from the group consisting of SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:54;
- when D14S118, at least one of the primers has a sequence selected from the group consisting of SEQ ID NO:17, SEQ ID NO:18;
- 5 when D14S562, at least one of the primers has a sequence selected from the group consisting of SEQ ID NO:19, SEQ ID NO:20;
- when D14S548, at least one of the primers has a sequence selected from the group consisting of SEQ ID NO:21, SEQ ID NO:22;
- when D16S490, at least one of the primers has a sequence selected from the group consisting of SEQ ID NO:23, SEQ ID NO:24;
- 10 when D16S753, at least one of the primers has a sequence selected from the group consisting of SEQ ID NO:25, SEQ ID NO:26;
- when D17S1299, at least one of the primers has a sequence selected from the group consisting of SEQ ID NO:27, SEQ ID NO:28;
- 15 when D16S539, at least one of the primers has a sequence selected from the group consisting of SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:58;
- when D22S683, at least one of the primers has a sequence selected from the group consisting of SEQ ID NO:31, SEQ ID NO:32;
- when HUMCSF1PO, at least one of the primers has a sequence selected from the group consisting of SEQ ID NO:33, SEQ ID NO:34;
- 20 when HUMTPOX, at least one of the primers has a sequence selected from the group consisting of SEQ ID NO:35, SEQ ID NO:36;
- when HUMTH01, at least one of the primers has a sequence selected from the group consisting of SEQ ID NO:37 SEQ ID NO:38;
- 25 when HUMvWFA31, at least one of the primers has a sequence selected from the group consisting of SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:60;
- when HUMF13A01, at least one of the primers has a sequence selected from the group consisting of SEQ ID NO:41, SEQ ID NO:42;
- 30 when HUMFESFPS, at least one of the primers has a sequence selected from the group consisting of SEQ ID NO:43, SEQ ID NO:44;

when HUMBFXIII, at least one of the primers has a sequence selected from the group consisting of SEQ ID NO:45, SEQ ID NO:46;

when HUMLIPOL, at least one of the primers has a sequence selected from the group consisting of SEQ ID NO:47, SEQ ID NO:48;

5 when D19S253, at least one of the primers has a sequence selected from the group consisting of SEQ ID NO:50, SEQ ID NO:51; and

when D4S2368, at least one of the primers has a sequence selected from the group consisting of SEQ ID NO:56, SEQ ID NO:57.

10 27. The kit of claim 25, further comprising a container having reagents for at least one multiplex amplification reaction.

15 28. The kit of claim 25, further comprising a container having an allelic ladder.

20 29. The kit of claim 28, wherein each rung of the allelic ladder and at least one oligonucleotide primer for each of the loci in the set each have a label covalently attached thereto.

30. The kit of claim 29, wherein the label is a fluorescent label.

25 31. The kit of claim 30, wherein at least one of the oligonucleotide primers has a different fluorescent label covalently attached thereto than some of the other primer pairs in the container.

30 32. A pair of oligonucleotide primers flanking locus D3S1539 in human DNA, wherein one primer of the pair has a sequence of SEQ ID NO:7, and wherein the other primer of the pair has a sequence selected from the group consisting of SEQ ID NO:8 and SEQ ID NO:49.

33. A pair of oligonucleotide primers flanking locus D17S1298 in human DNA, wherein one primer of the pair has a sequence of SEQ ID NO:9, and wherein the other primer of the pair has a sequence of SEQ ID NO:10.

5 34. A pair of oligonucleotide primers flanking locus D20S481 in human DNA, wherein one primer of the pair has a sequence selected from the group consisting of SEQ ID NO:11 and SEQ ID NO:52, and wherein the other primer of the pair has a sequence selected from the group consisting of SEQ ID NO:12 and SEQ ID NO:53.

10 35. A pair of oligonucleotide primers flanking locus D9S930 in human DNA, wherein one primer of the pair has a sequence of SEQ ID NO:55, and wherein the other primer of the pair has a sequence selected from the group consisting of SEQ ID NO:14 and SEQ ID NO:61.

15 36. A pair of oligonucleotide primers flanking locus D4S2368 in human DNA, wherein one primer of the pair has a sequence of SEQ ID NO:56 and wherein the other primer of the pair has a sequence of SEQ ID NO:57.

20 37. A method of simultaneously determining the alleles present in at least four short tandem repeat loci from one or more DNA samples, comprising:

- a. obtaining at least one DNA sample to be analyzed,
- 25 b. selecting a set of at least four short tandem repeat loci of the DNA sample to be analyzed which can be amplified together, wherein three of the loci in the set are D7S820, D13S317, and D5S818;
- c. co-amplifying the set of loci in a multiplex amplification reaction, wherein the product of the reaction is a mixture of amplified alleles
- 30 from each of the co-amplified loci in the set; and

d. evaluating the amplified alleles in the mixture to determine the alleles present at each of the loci analyzed in the set within the DNA sample.

5 38. The method of claim 37, wherein the multiplex amplification reaction is done using at least four pair of primers flanking the at least four loci analyzed.

10 39. The method of claim 38, additionally comprising the step of selecting pairs of primers for the multiplex amplification reaction which produce alleles from each locus that do not overlap the alleles of the other loci in the set co-amplified therein, when the alleles are separated by gel electrophoresis.

15 40. The method of claim 37, wherein the multiplex amplification reaction is a polymerase chain reaction.

20 41. The method of claim 37, wherein the amplified alleles are evaluated by comparing the amplified alleles to a size standard, wherein the size standard is selected from the group of size standards consisting of a DNA marker and a locus-specific allelic ladder.

25 42. The method of claim 37, wherein the amplified alleles are evaluated using polyacrylamide gel electrophoresis to separate the alleles, forming a polyacrylamide gel of separated alleles.

30 43. The method of claim 42, wherein the separated alleles in the polyacrylamide gel are determined by visualizing the alleles with silver stain analysis.

44. The method of claim 42, wherein primers capable of binding to a region flanking each of the loci in the set are used in co-amplifying the loci, wherein at least one of the primers used in co-amplifying each locus has a fluorescent label covalently attached thereto such that the amplified alleles
5 produced therefrom are fluorescently labeled, and wherein the separated alleles in the polyacrylamide gel are determined by visualizing the alleles with fluorescent analysis.

45. The method of claim 44, wherein the fluorescent label is
10 selected from the group of labels consisting of fluorescein and tetramethyl rhodamine.

AMENDED SHEET (ARTICLE 19)

STATEMENT UNDER PCT ARTICLE 19:

Applicant respectfully submits that none of the amendments to any of the claims presented herein adds any new matter to the subject international application. The amendments are introduced herein to clarify the subject matter of each claim, as filed, and to facilitate examination of the application by the International Search Authority.

Applicant hereby requests communication of the enclosed PCT Article amendments to the Designated Offices, in accordance with PCT Article 20(2).

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